Nanotoxicology as a Predictive Science that can be explored by high content screening and the use of computer-assisted hazard ranking

André Nel M.B.,Ch.B; Ph.D
Professor of Medicine and Chief of the Division of NanoMedicine at UCLA

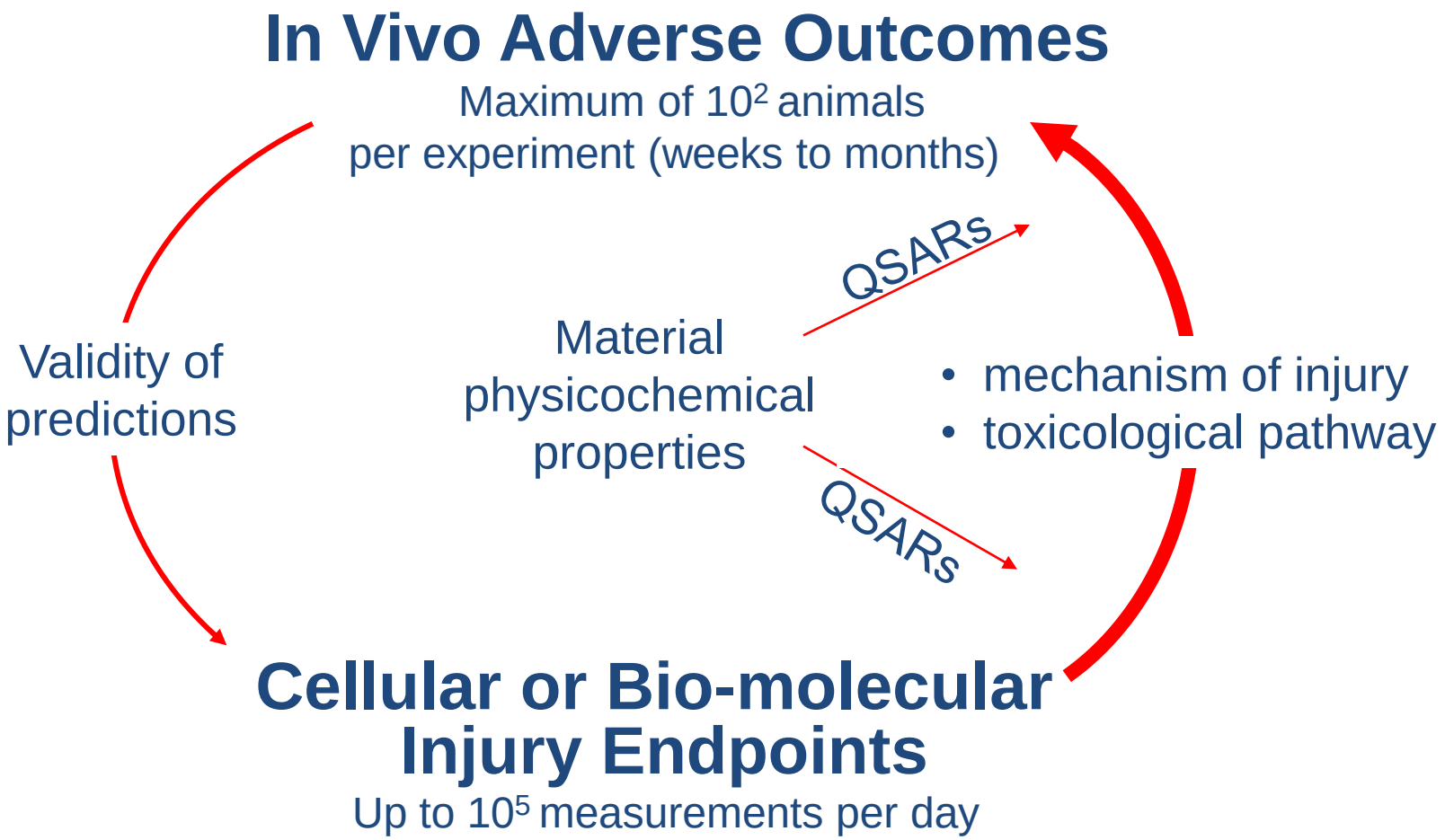
Director of the NSF- and EPA-funded Center for the Environmental Implications of Nanotechnology (UC CEIN)

Director of the NIEHS-funded Center for NanoBiology and Predictive Toxicology

This materials is based on work supported by the National Science Foundation and Environmental Protection Agency under Cooperative Agreement # NSF-EF0830117. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the Environmental Protection Agency.

Proposal

Predictive Toxicological Paradigm to resolve the safety assessment of a large number of nanomaterials



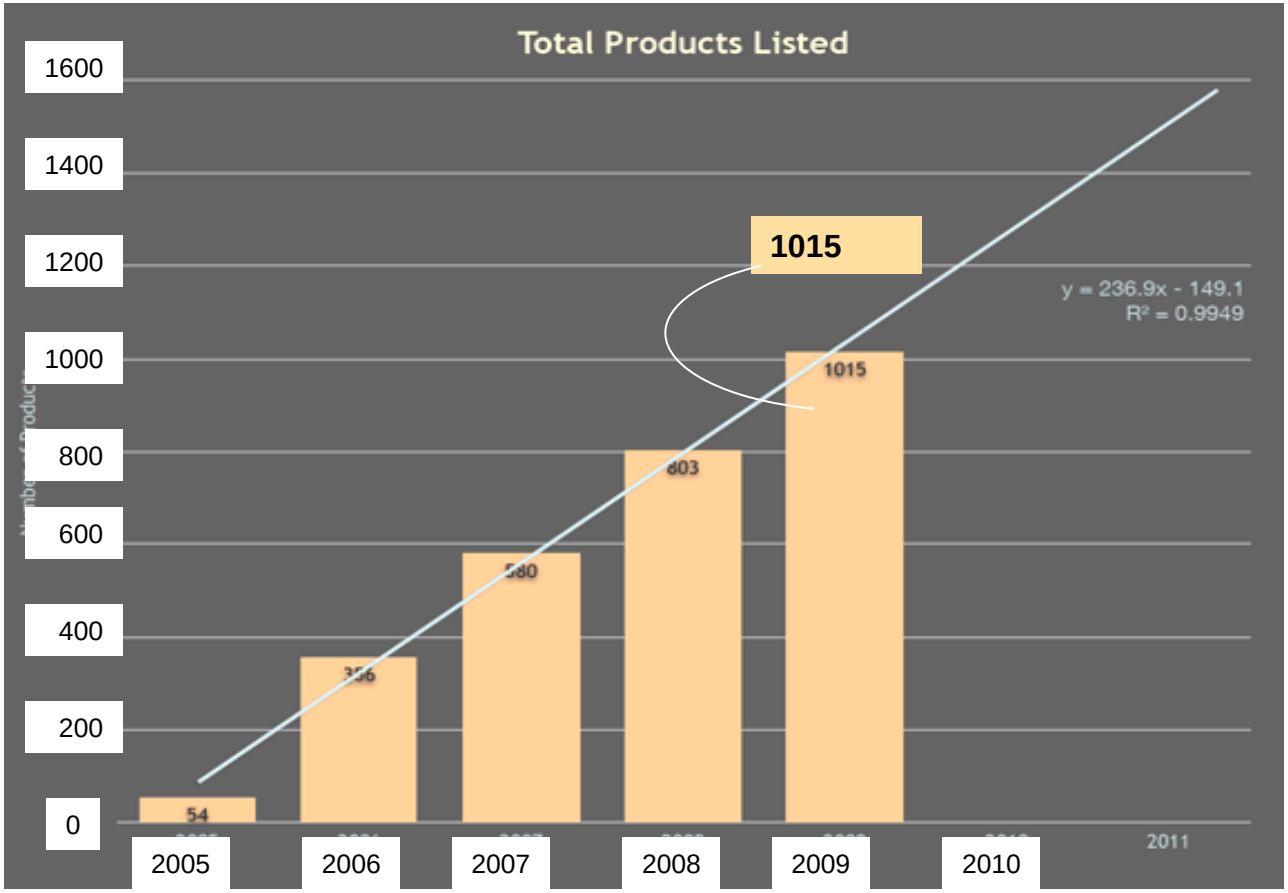
Nel et al Science 2006.

Huan Meng et al ACS Nano. 2009

Talk Outline

1. Why do we need a predictive science for hazard ranking and decision making?
2. What exactly are the linkages that connect the *in vitro* knowledge domain to *in vivo* hazard assessment?
3. What is the appropriate place for high content or high throughput screening and nano informatics in a predictive hazard platform?
4. What other benefits are there in using a predictive toxicology approach?

Prolific Growth of Nanotechnology



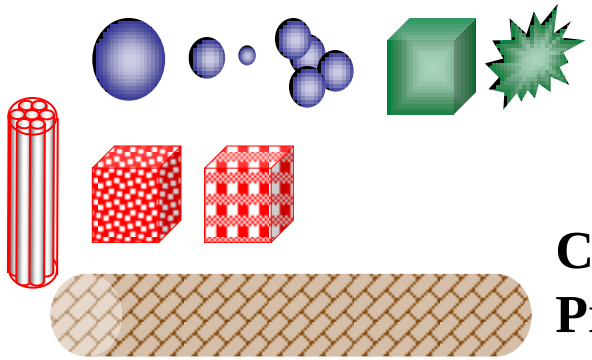
Source: Project on Emerging Nanotechnologies

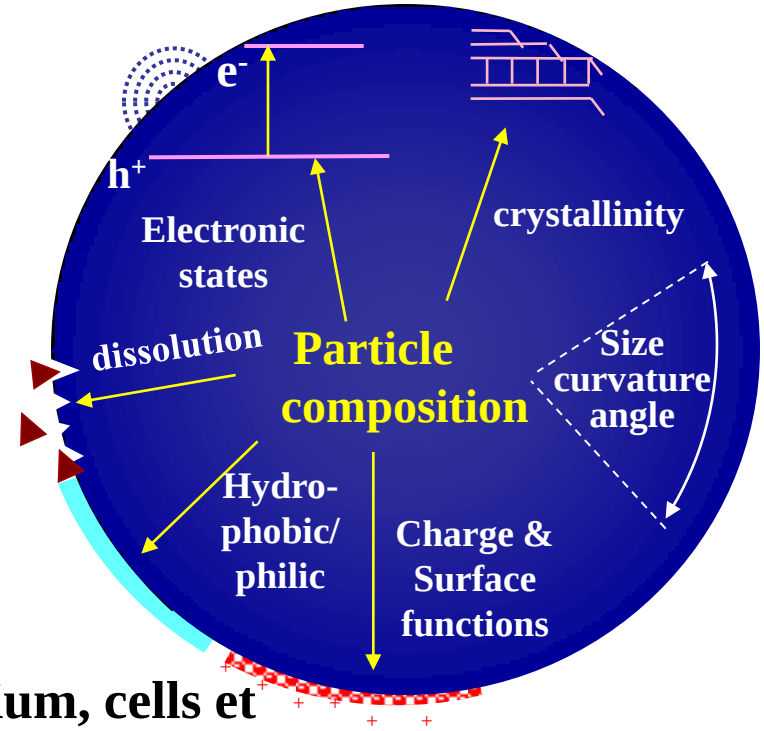
Why do we need a Predictive Science for ENM hazard assessment?

1. Need for a hazard platform that keeps pace with the rate at which new ENMs are being introduced
2. It is impossible to use animal testing as the primary platform for ENM hazard assessment
3. Need for an *in vitro* property-activity approach that utilizes high-volume data collection to prioritize and speed up *in vivo* decision-making
4. Need for a robust scientific platform to link *in vitro* to toxicological relevant *in vivo* outcomes (will propose mechanistic toxicological pathways)

A large number of material properties and bio-interfaces generate an endless number of possibilities at the nano-bio interface

Different sizes/shapes/aspect ratios
States of agglomeration
Media interactions
etc etc





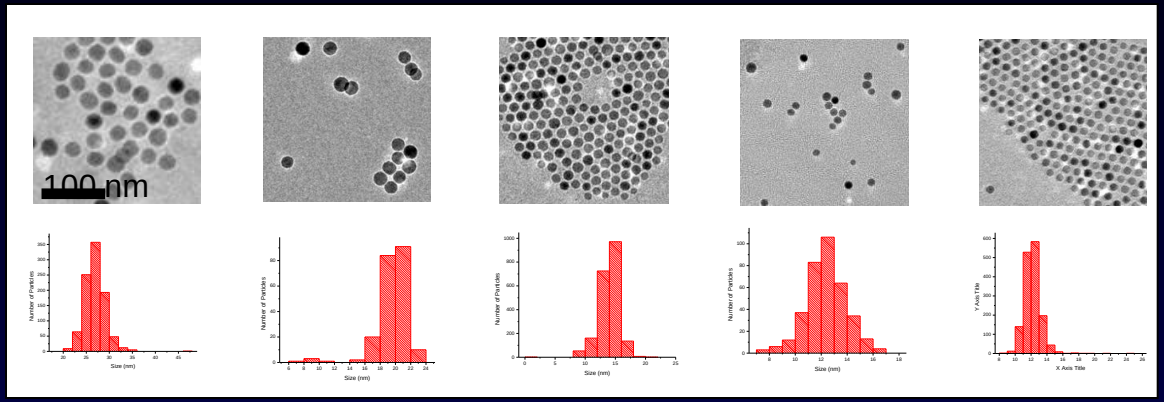
Cell membrane
Proteins in the medium, cells et
DNA and nucleus
Cell uptake (endocytosis/phagocytosis etc)
Subcellular localization/organellar interactions
Mitochondrial functions/ATP production
Bio-accumulation/biopersistance
etc etc

Nel et Nature Mat. 2009

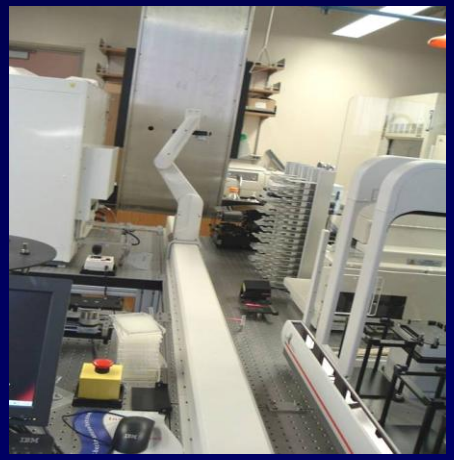
It’s All So Complex but ..

(d)

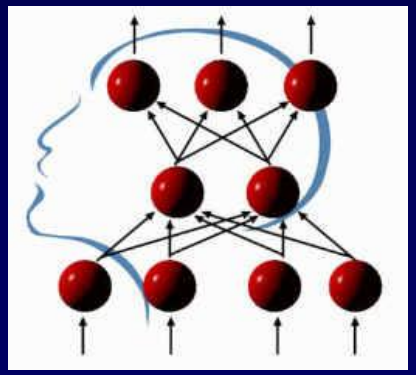
We can track NP and use their properties



We can make model libraries of materials



We can use high throughput approaches



We can use computational power to enhance and speed up decision-making

We can use property-activity relationships to make safer materials or prevent exposure to materials with hazardous properties

US National Academy of Science's Recommended Transformative approach *"Toxicity Testing in the 21st Century: A Vision and a Strategy" (2007)*

- Provide wide coverage of potential toxicants
- Use a robust scientific base for testing (instead of a descriptive approach in whole animals)
- Comprehensive array of predictive *in vitro* tests that utilize toxicity pathways and mechanisms
- High content or high throughput screening to facilitate testing of large batches of materials
- *In vitro* hazard to be confirmed *in vivo*

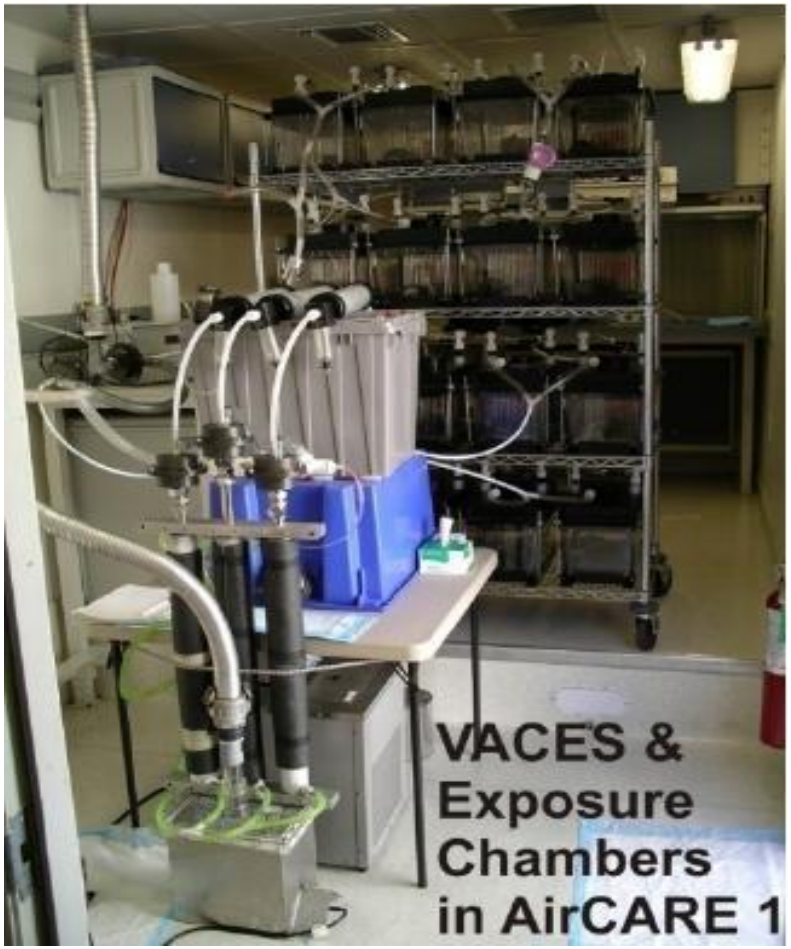
http://www.nap.edu/catalog.php?record_id=11970

NP toxicological considerations covered in this talk

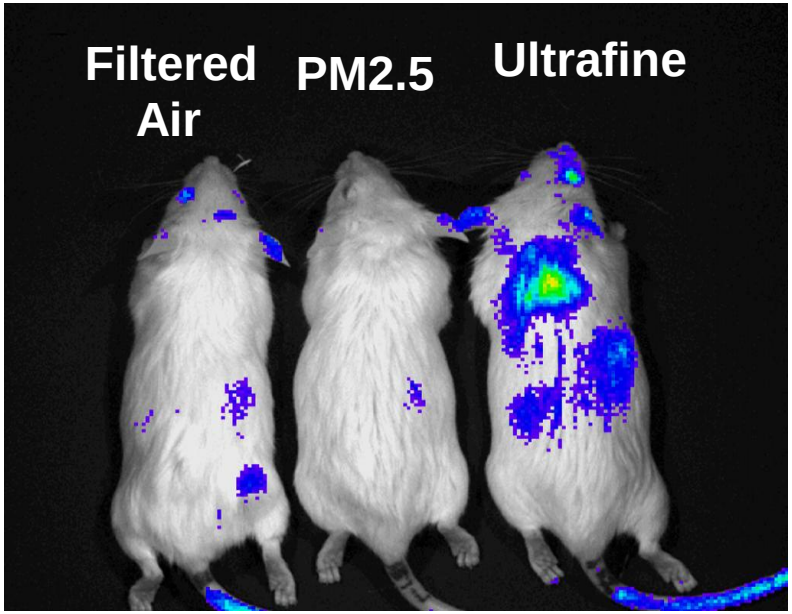
1. Predictive pulmonary toxicity

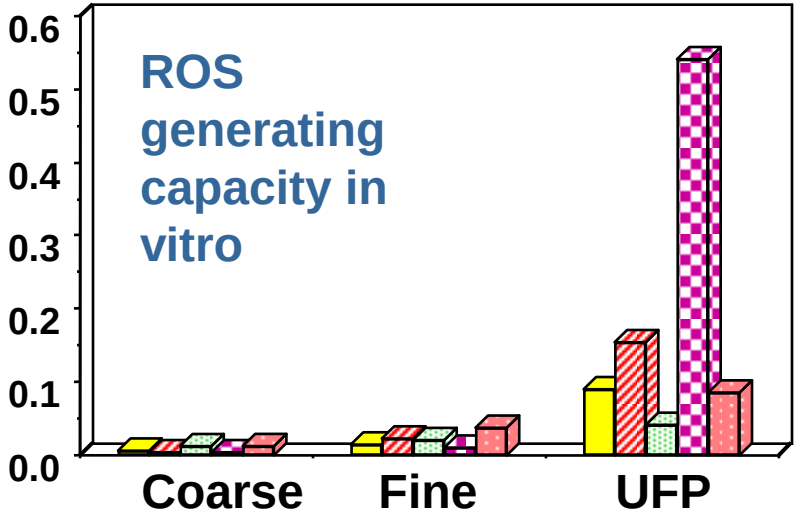
2. Predictive environmental toxicology , e.g., zebrafish

Predictive toxicology in air pollution research

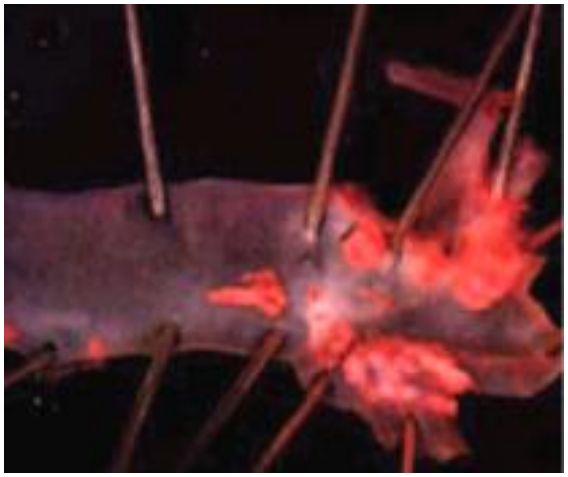


Oxidative Stress as a Predictive Toxicological Paradigm: Real-life Proof that in vitro assessment of oxidant potential is linked to the exaggerated cardiovascular effects of ultrafine particles



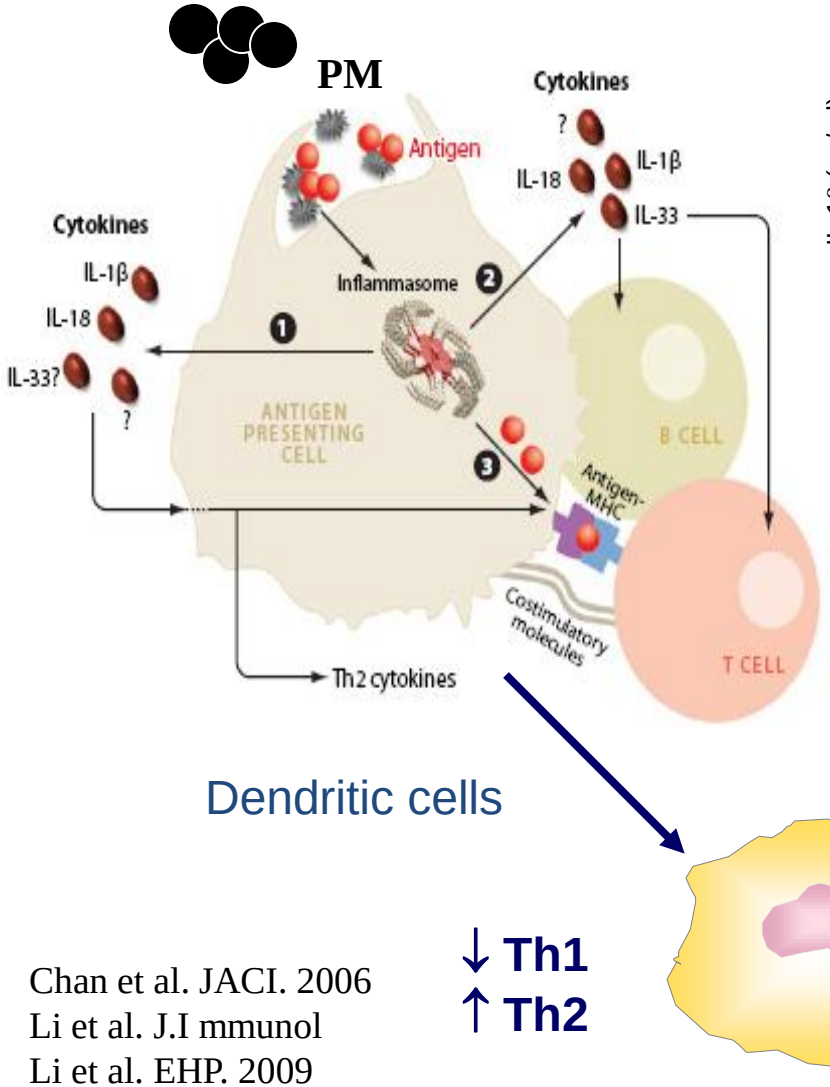


Generate Oxidant Injury in the Lung and Cardiovascular system (the lung signal is from an oxidative stress gene that is turned in a live animal exposed on an LA freeway)

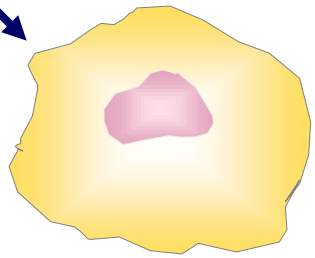


Increased rate of atherosclerosis in UFP compared to PM2.5 exposures

Oxidative Stress as a Predictive Toxicological Paradigm: Use of the mouse asthma model to demonstrate that ultrafine oxidant potential is linked to allergic sensitization

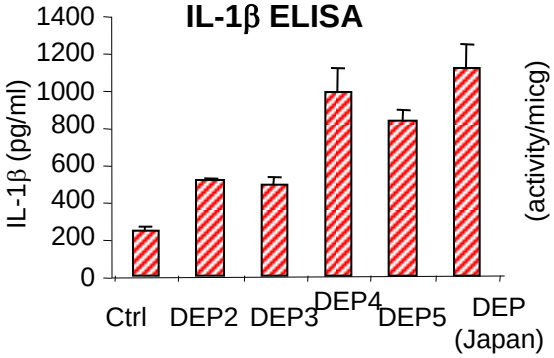


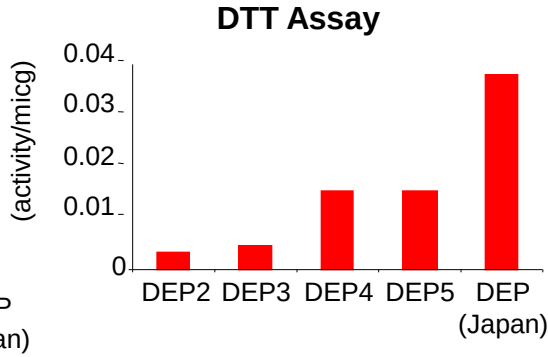
Dendritic cells

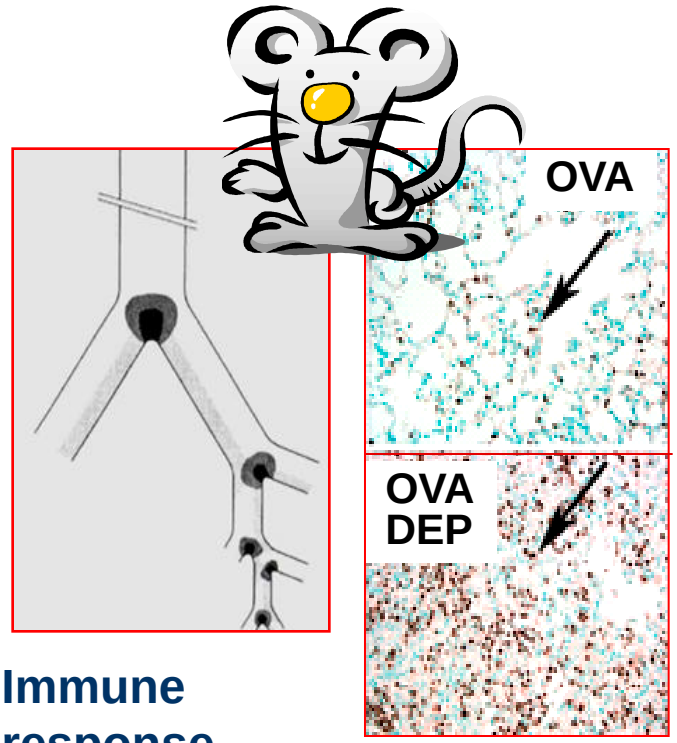


↓ Th1
↑ Th2

Chan et al. JACI. 2006
Li et al. J.I mmunol
Li et al. EHP. 2009

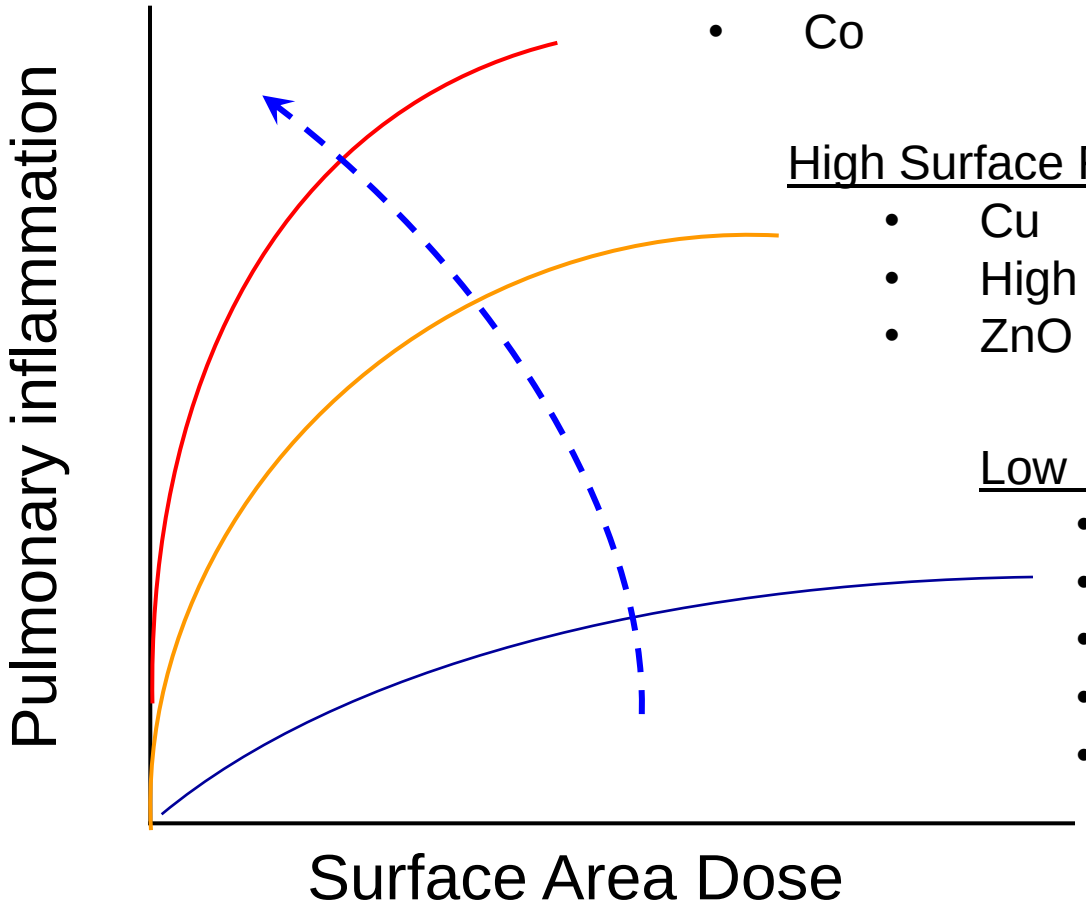






Immune response

A proposed paradigm for ENM pulmonary toxicity evaluation: Concept of NP Surface Reactivity



Very High Surface Reactivity:

- Crystalline Si (quartz)
- Ni
- Co

High Surface Reactivity:

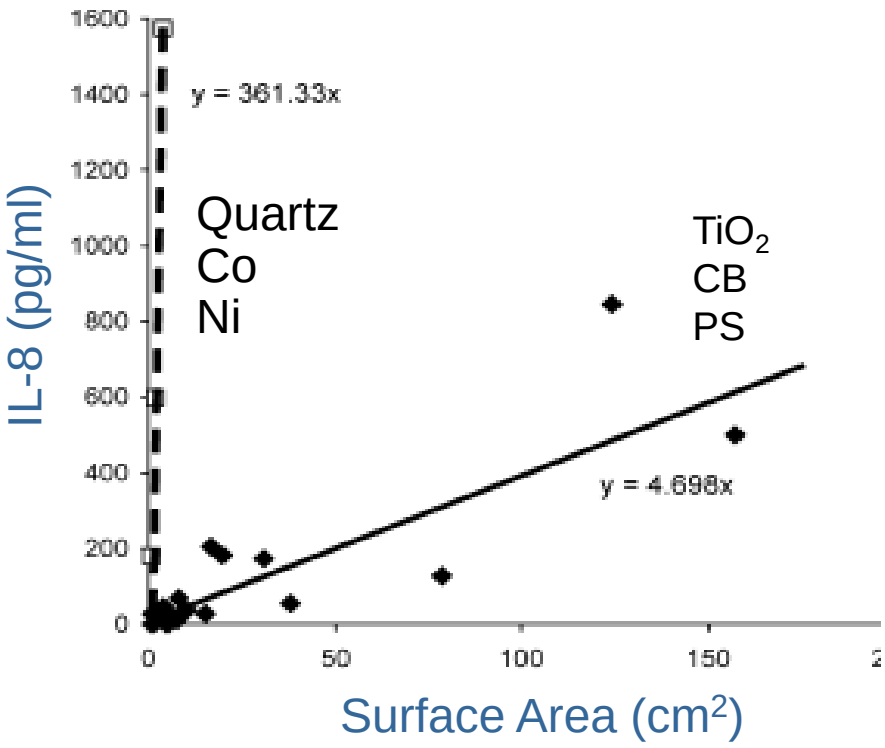
- Cu
- High cationic charge
- ZnO

Low Surface Reactivity:

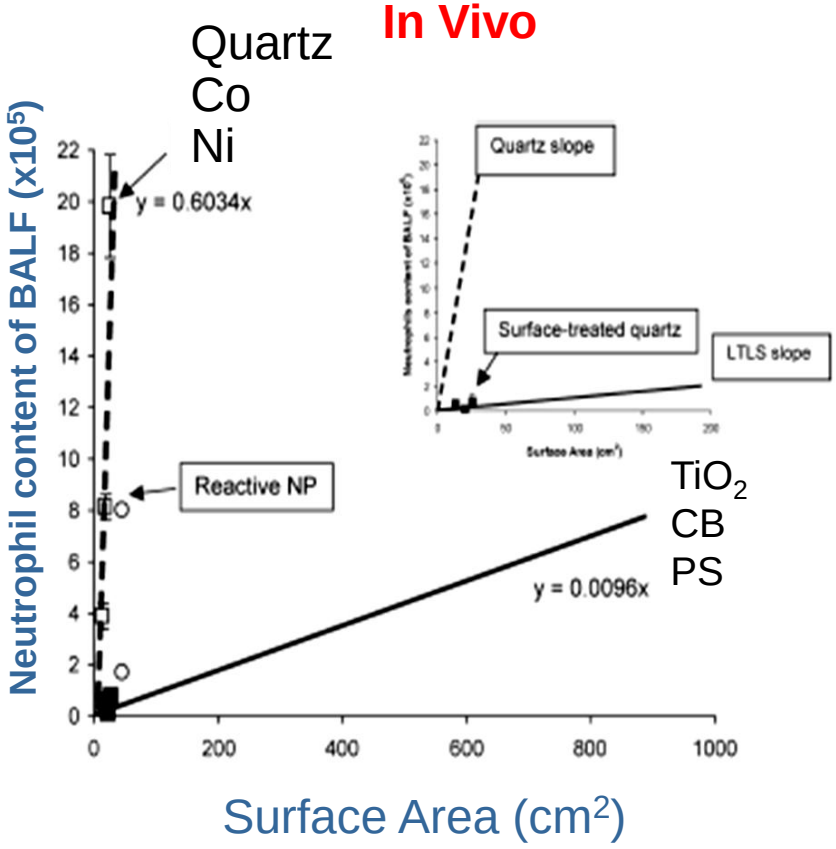
- TiO_2
- Au
- Carbon black
- Amorphous Si
- Polystyrene

Example: When Using IL-8 production in a bronchial epithelial cell line to discern between the inflammation potential of of High versus Low Reactive Surface Area NP in rats

In Vitro



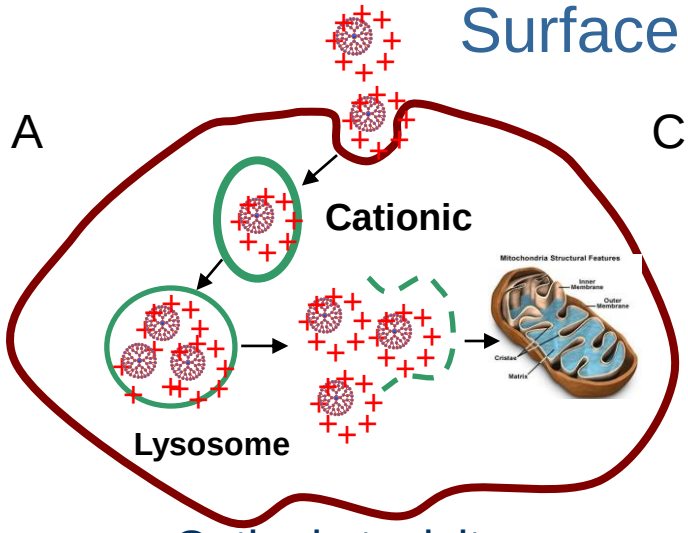
In Vivo



Adapted from Duffin et al Inhal Tox 2007

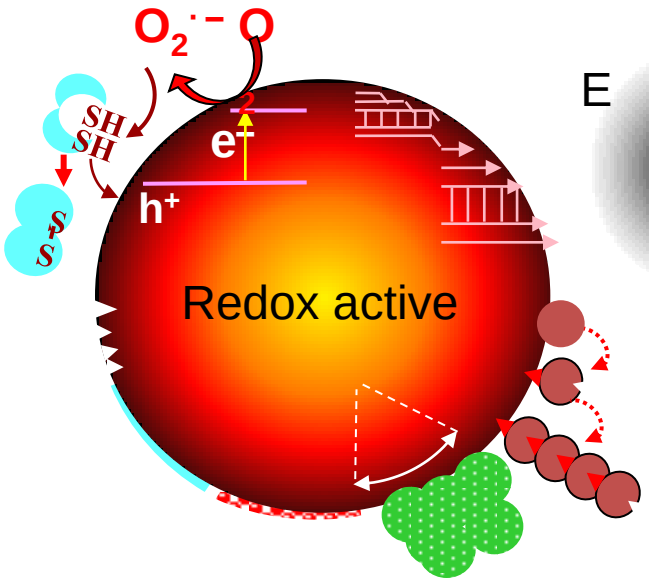
Surface Reactivity Paradigms

A

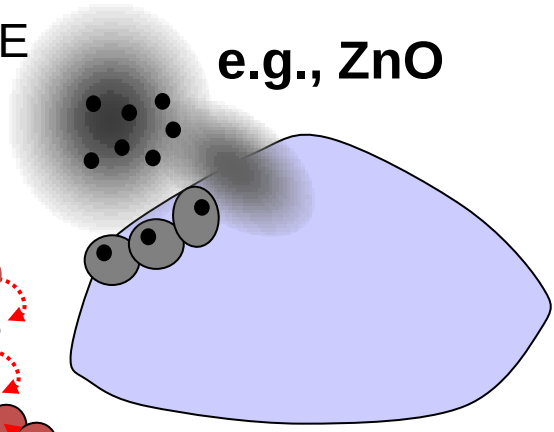


Cationic toxicity
Lysosomal, surface membrane

C

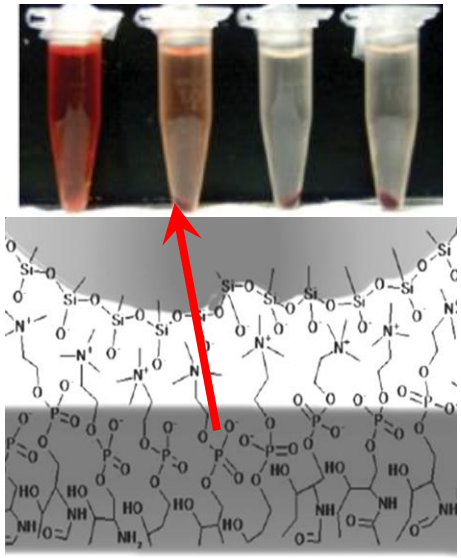


E



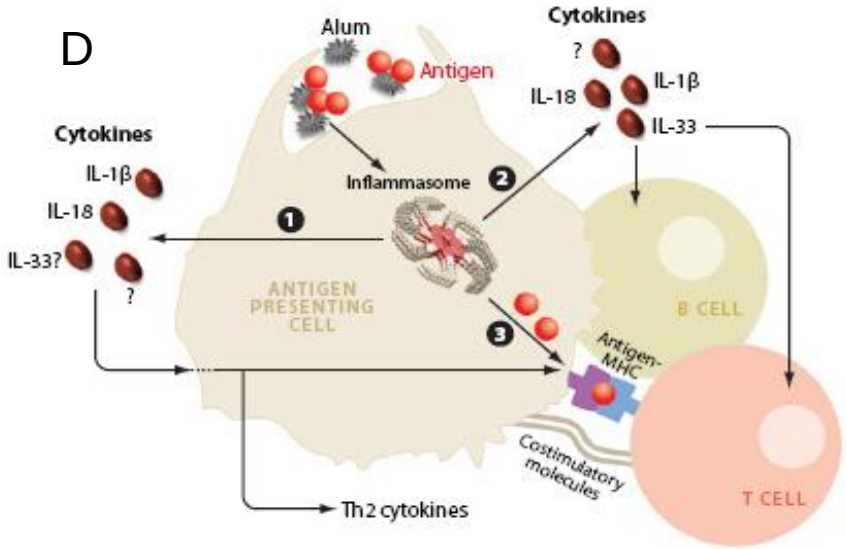
Dissolution and
Ion release

B



Membrane
Lysis, e.g, charge

D



Immune danger signals,
inflammazones
e.g., dendritic cells

Nel et al. Science. 2006
Nel et al. Nature Materials. 2009

Metal Fume Fever: Metal oxide toxicity

Welders exposed to ZnO, other metal oxides: Cu, Mg, Sn, or Cd

3-10 hrs post-exposure: flu-like illness, fever, malaise, chills, dry cough, shortness of breath

BAL cytokines:TNFα, IL-6, IL-8, MIP

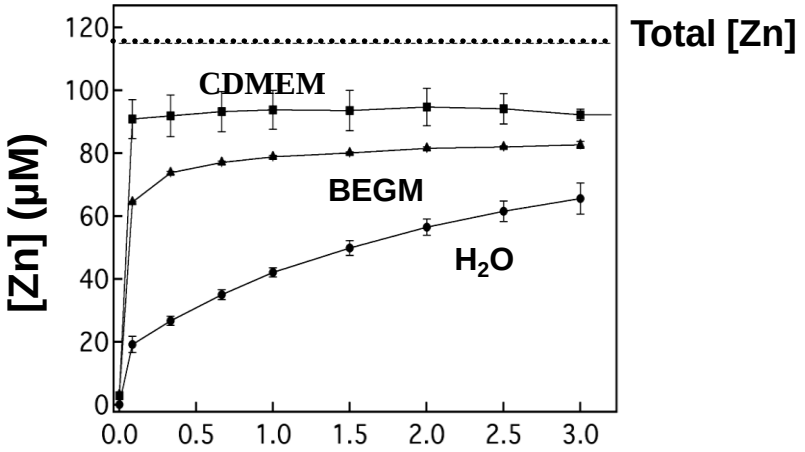
Pathophysiology: marked increases in lung PMLs 20–24 hr after exposure

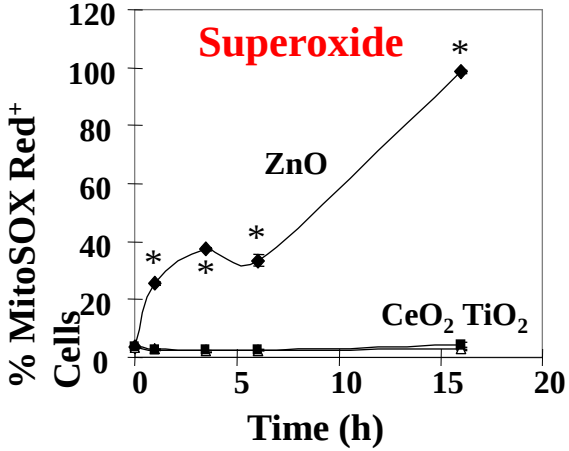
Resolves 24–48 hr after onset, no structural damage

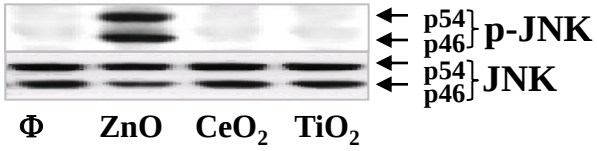
Short-term tolerance: asymptomatic with repeated exposure

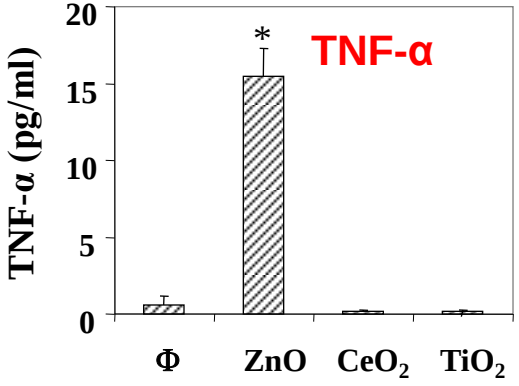
ZnO dissolution chemistry and cellular toxicity

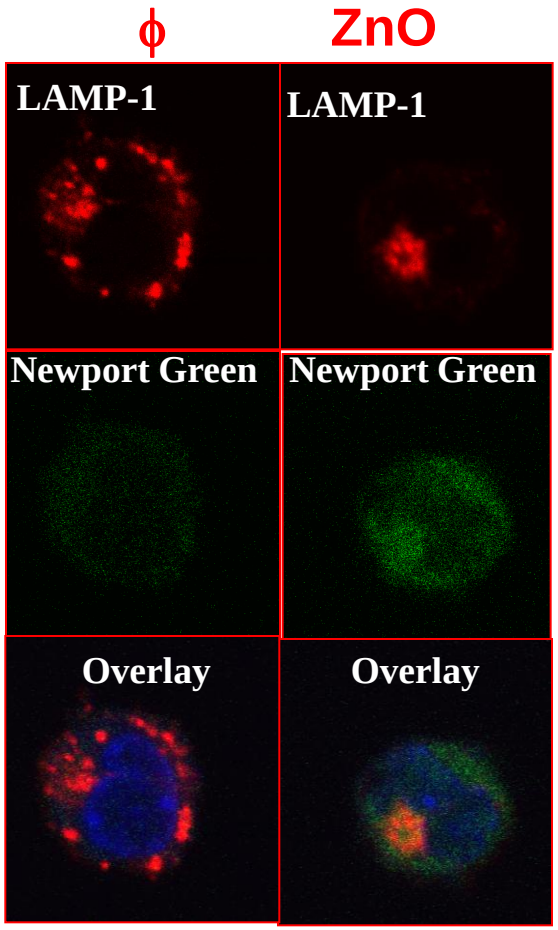
Xia et al
ACS Nano

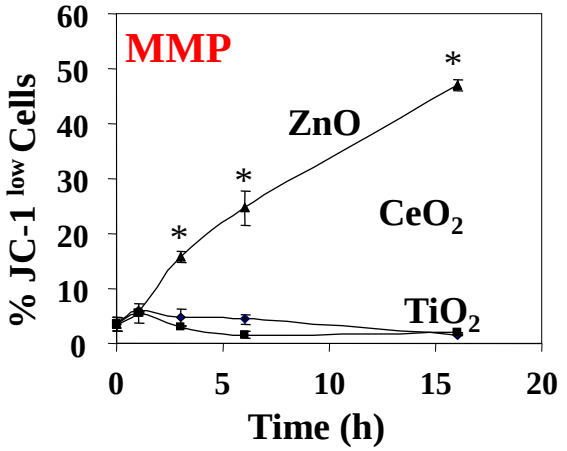


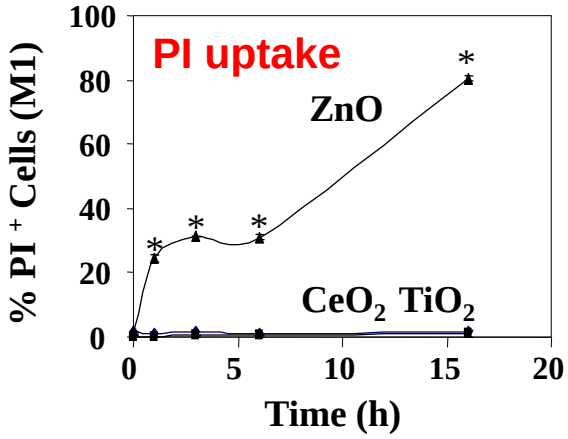




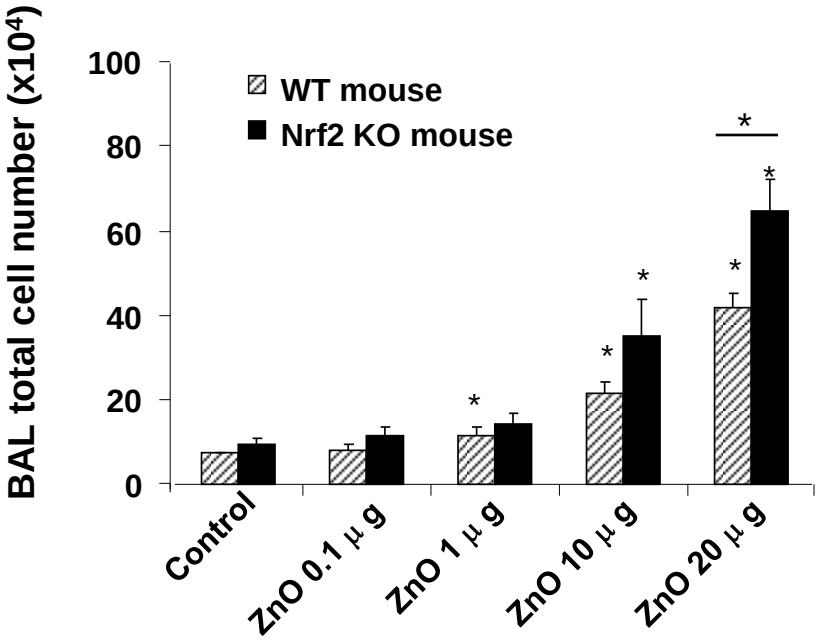


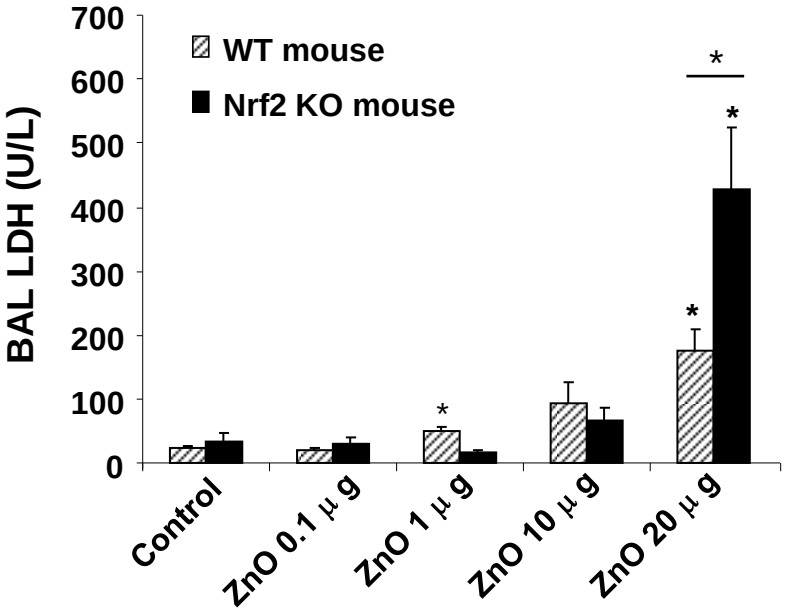


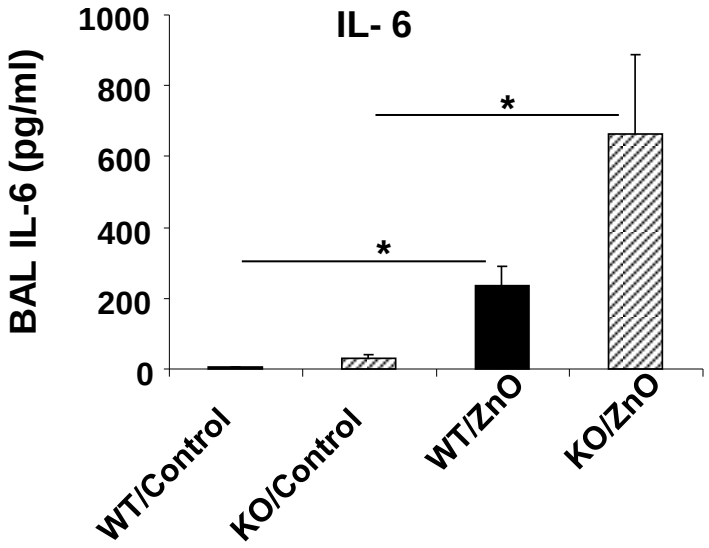




Mouse studies showing *in vivo* linkage to cellular effects





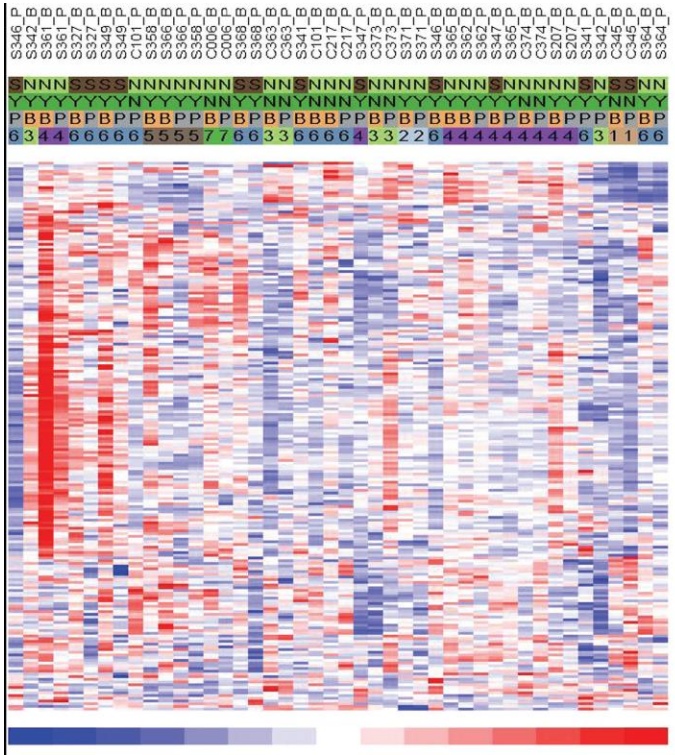


Human Research Data in welders showing connection of mechanistic cellular data to in vivo oxidative stress effects and inflammation

Microarray analysis of whole blood total RNA in boilermakers before and after occupational exposure to metal fumes

Genes with altered expression were clustered in biologic groupings that reflect induction of:

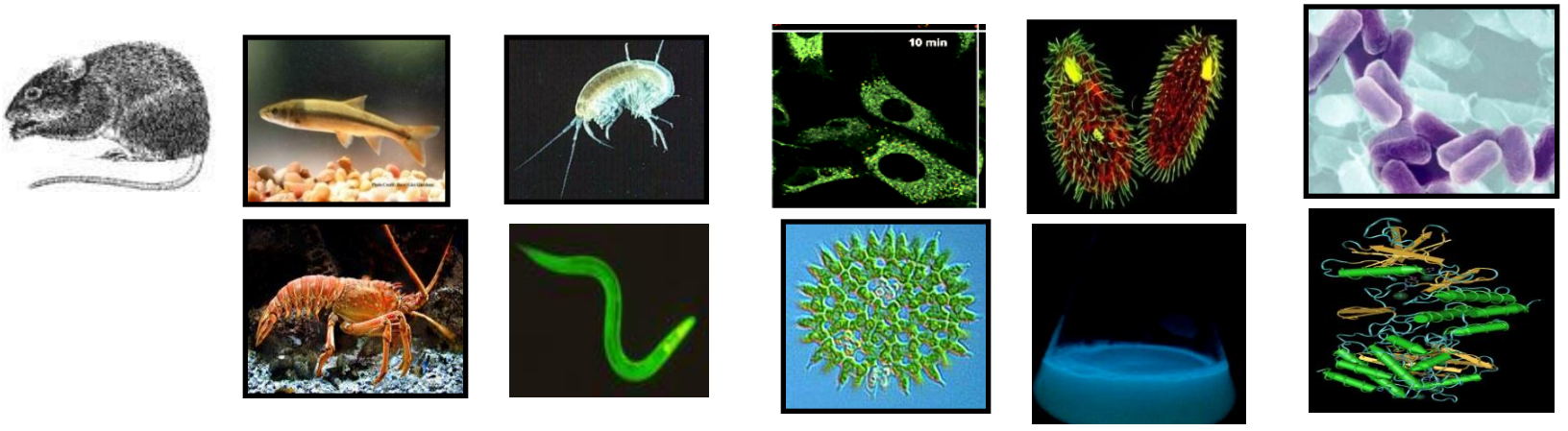
- inflammatory responses: esp IL-8
- oxidative stress
- signal transduction
- programmed cell death



Wang Z et al EHP 113:233–241 (2005).

NSF: EF-0830117

Predictive approach to environmental hazard assessment

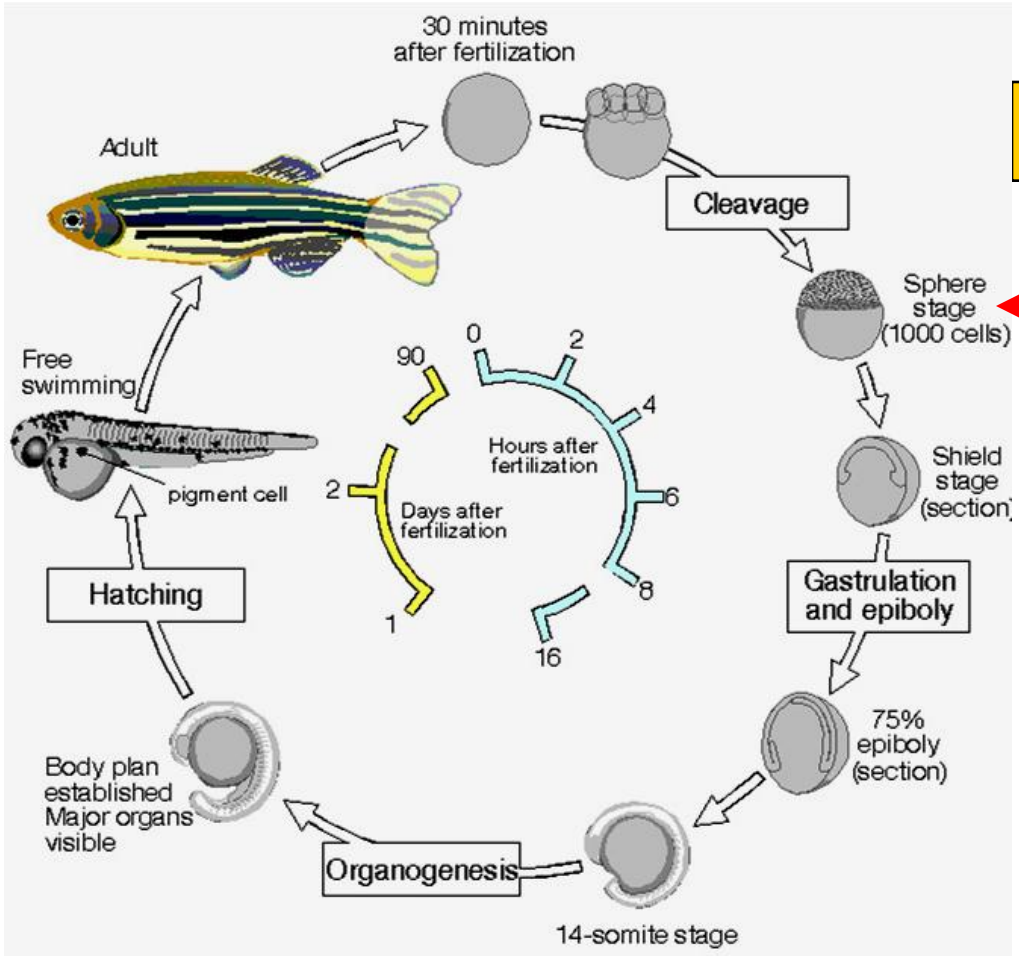


100's/year **1000's/year** **10,000's/day** **100,000's/day**

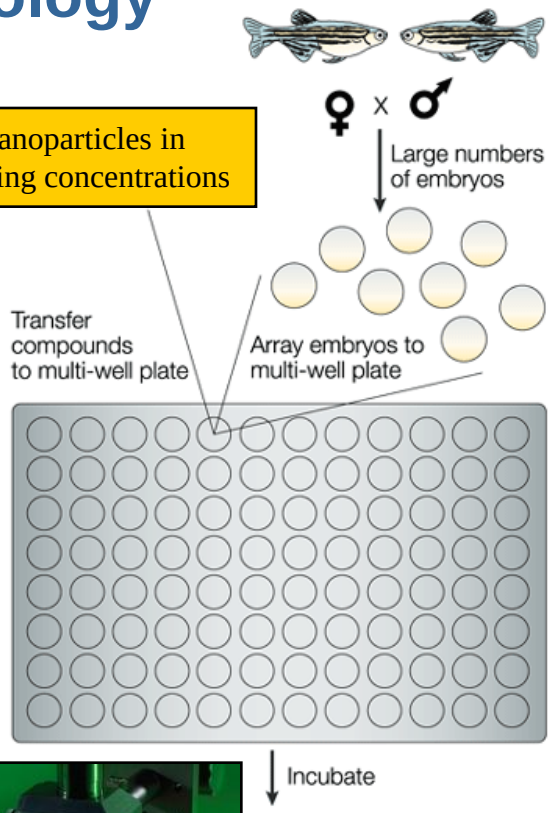
High Throughput Bacterial, Cellular, Yeast, Embryo or Molecular Screening

Prioritize *in vivo* testing at increasing trophic levels

Use of a Zebra fish model to perform Predictive Environmental Toxicology



Nanoparticles in working concentrations

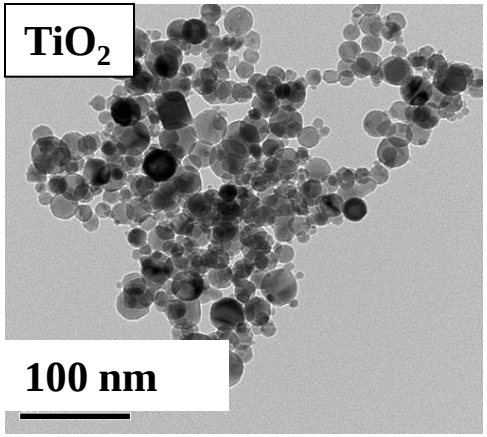


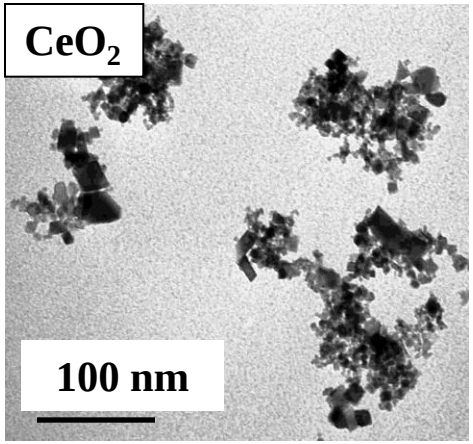
Observe and score for mortality rate, hatching rate, morphology and physiology

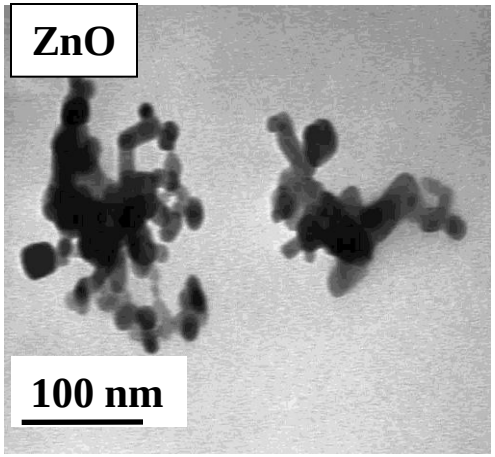
UCLA UCDAVIS BODEGA MARINE LABORATORY

David Schoenfeld, Tian Xia, Saji George, Ivy Ji, Shou Lin, Andre Nel

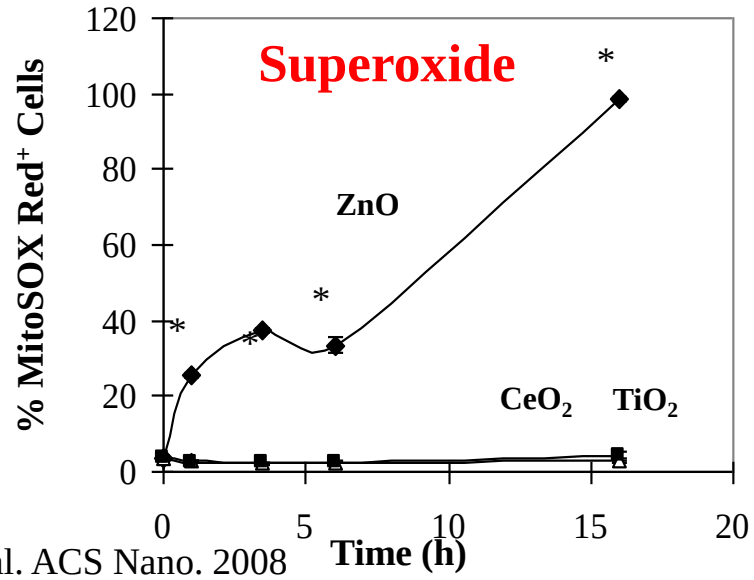
Comparison three MeO *in vitro*



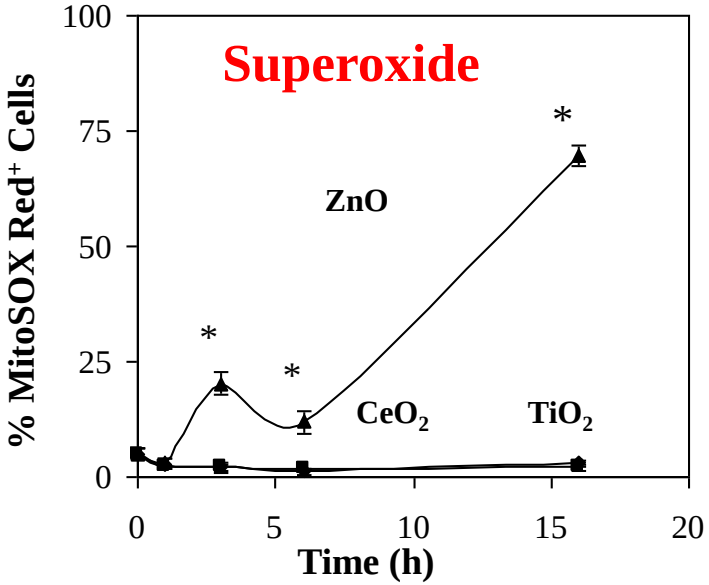




Macrophage line

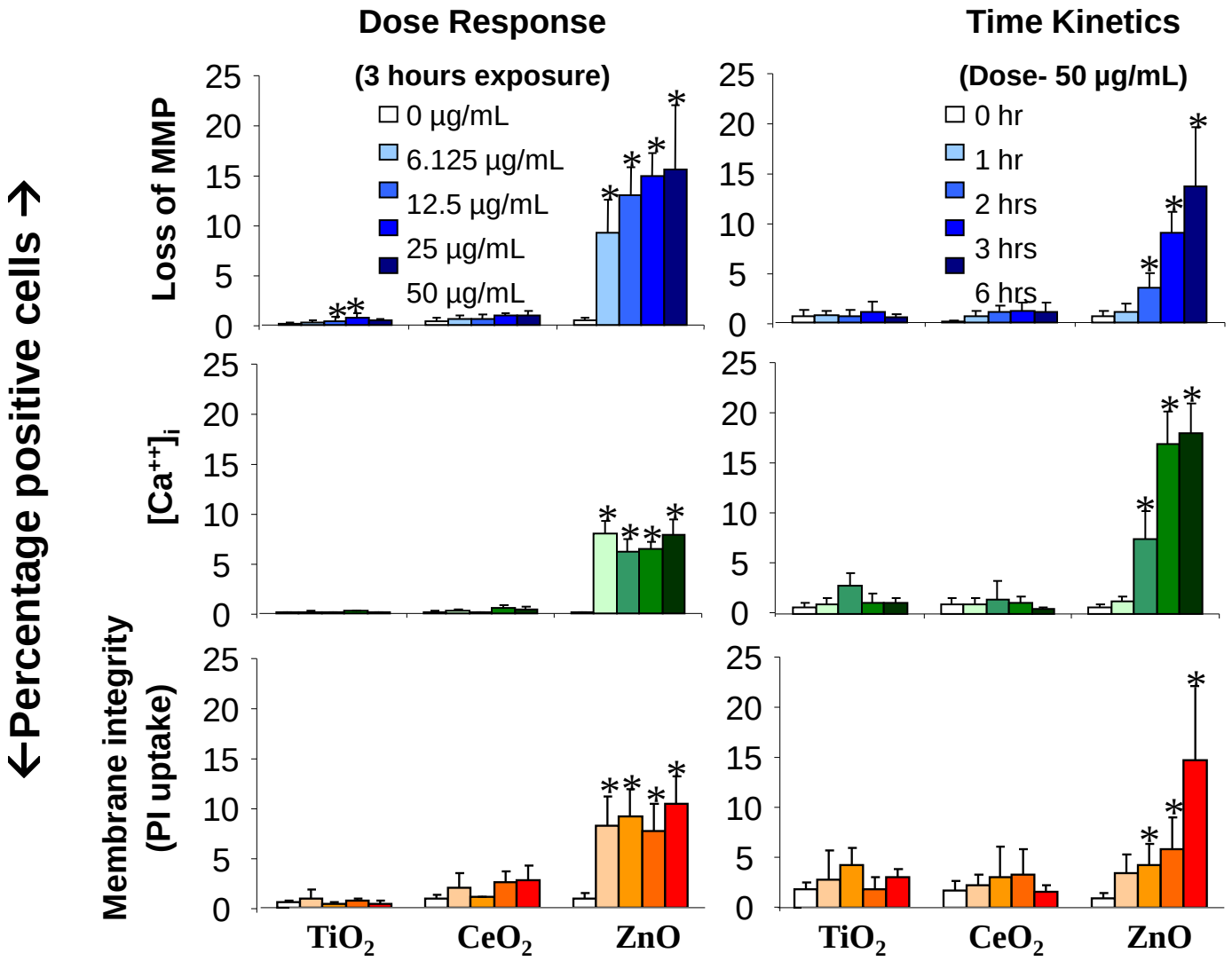


Epithelial line



Xia et al. ACS Nano. 2008

Dose- and time- dependent rapid throughput cytotoxicity assay in BEAS-2B cells

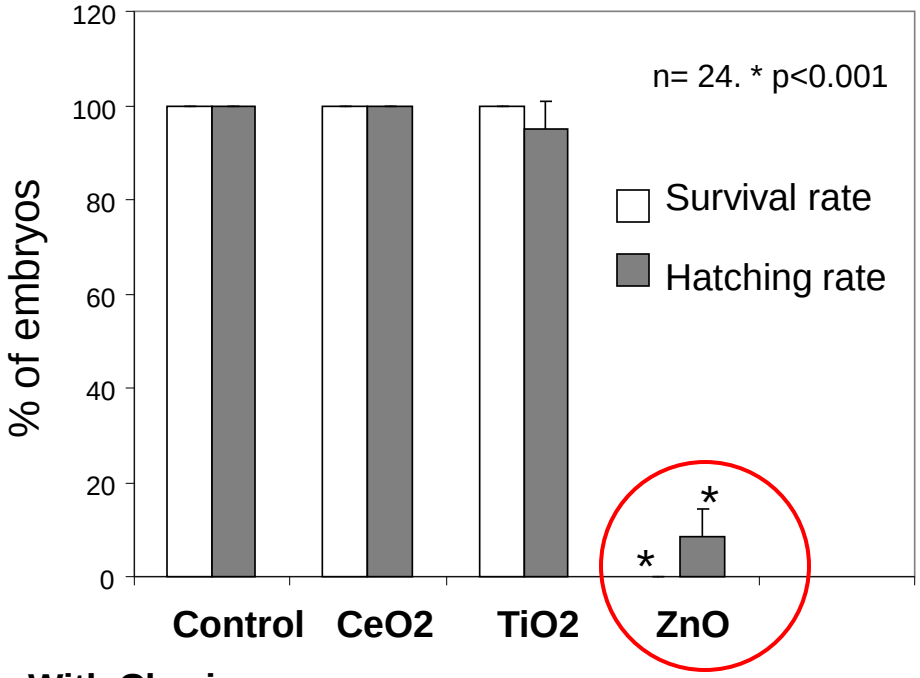


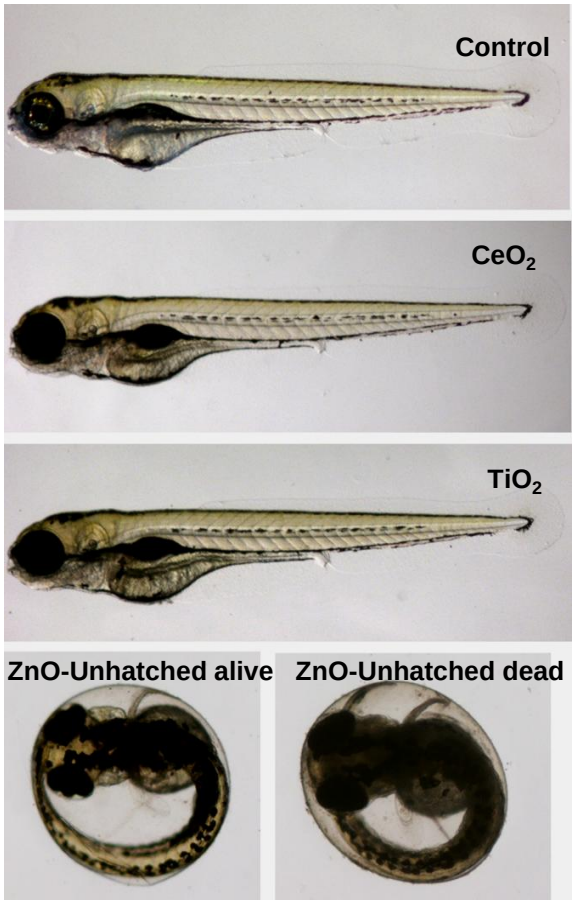
- ZnO showed dose and time dependent increase in all parameters of toxicity
- TiO_2 and CeO_2 showed no cytotoxicity

George, Xia, Nel, et al. ACS Nano, 2010, 4, 15–29

UCLA

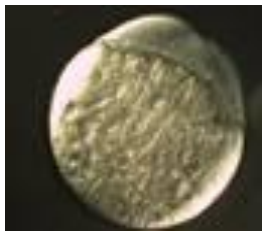
Comparing the toxicity of the MeO library in Zebrafish

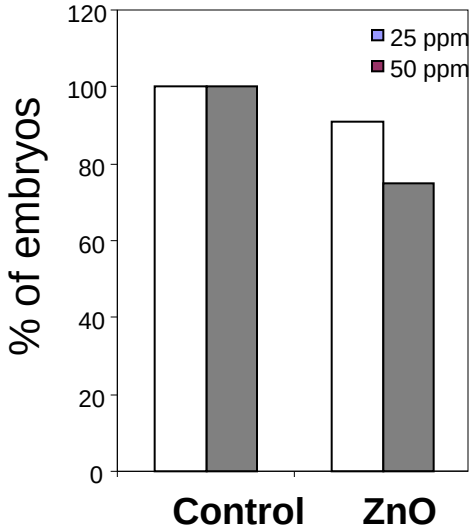


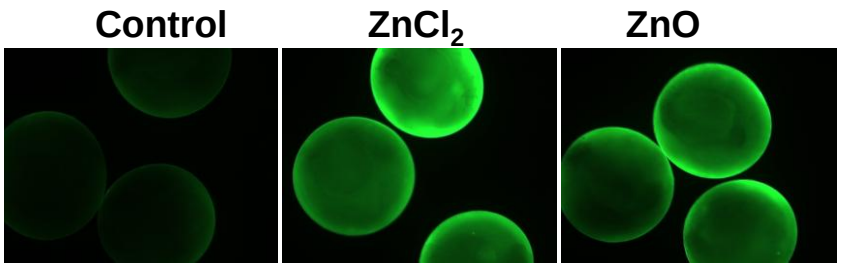


With Chorion

Without Chorion







What are the key ingredients for establishing a predictive science at the nano-bio interface?

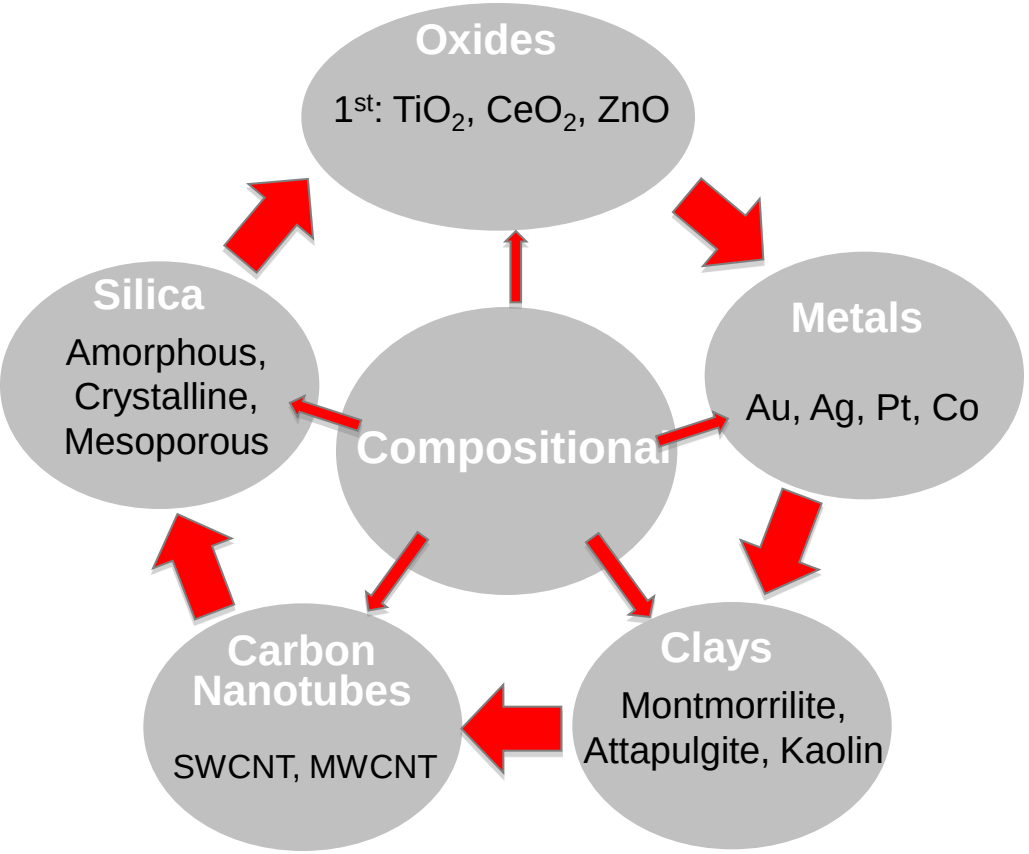
1. The development of appropriate cellular and bio-molecular assays that can be used for predicting ENM hazard in intact animals

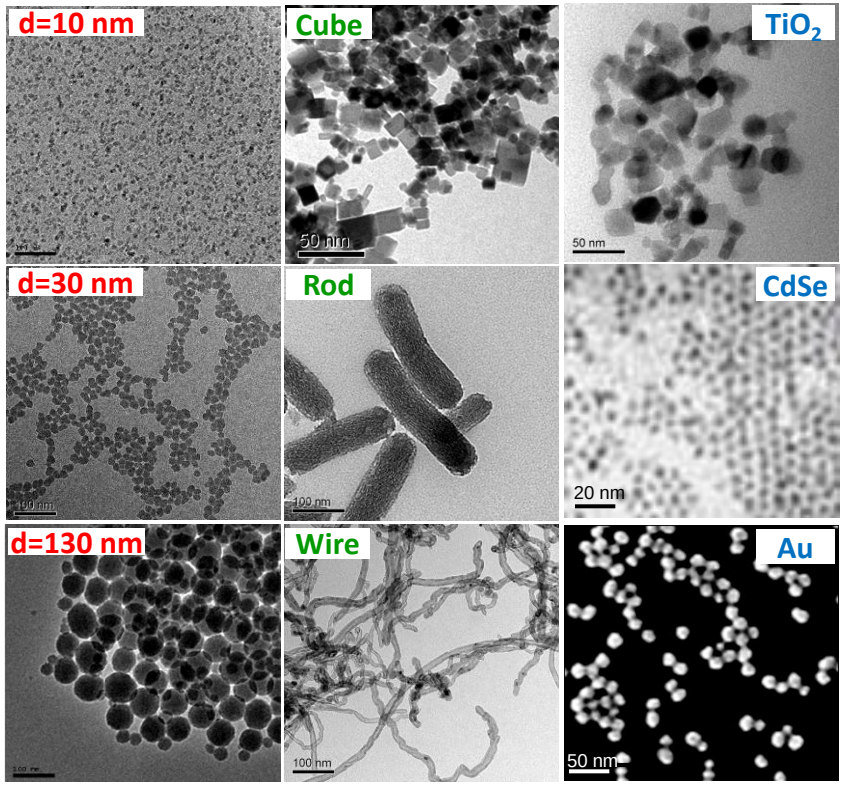
2. Development of compositional and combinatorial ENM libraries that can be used to explore property-activity relationships

4. Ability to perform high throughput screening to speed up knowledge generation

5. Computational analysis and nano-bioinformatics to deal with high volume data sets and ability to make predictions

The use of Compositional and Property-based Nanomaterial Libraries to make discoveries at the nano-bio interface



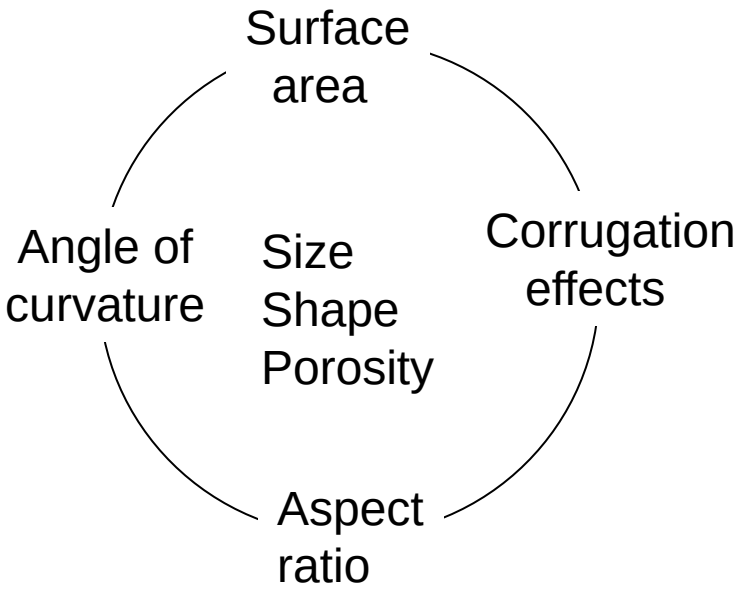


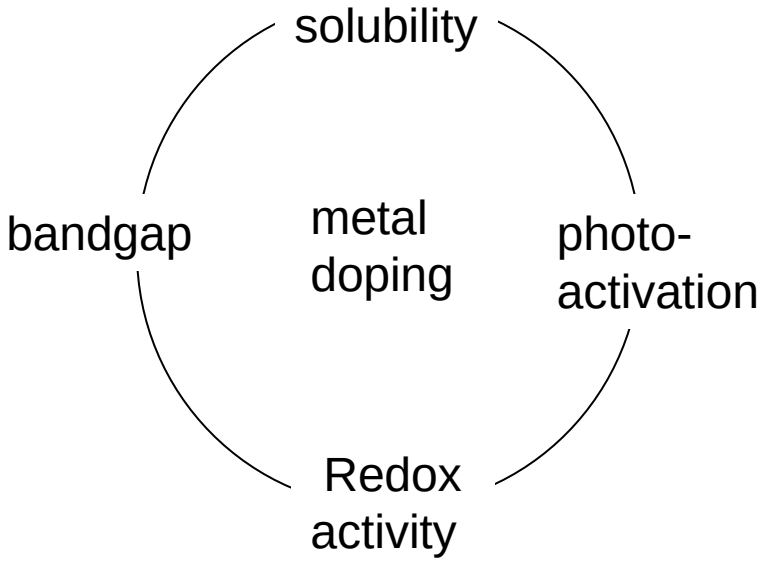
Size **Shape** **Composition**

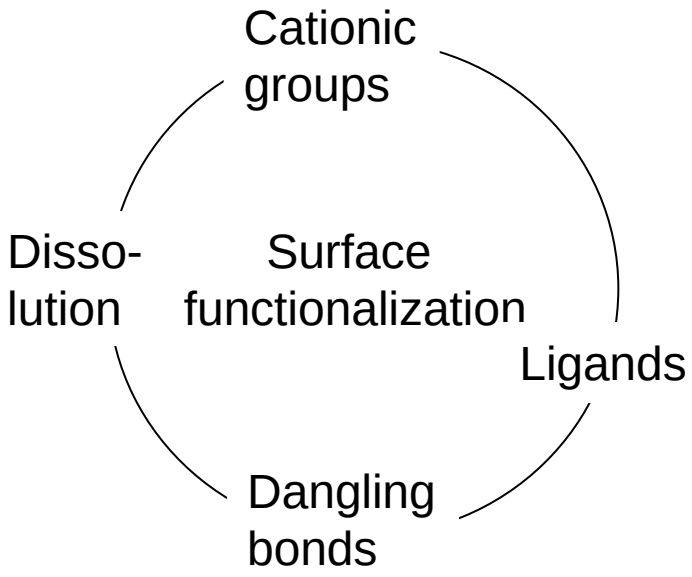
*Group Leader: **Jeff Zink (UCLA)***

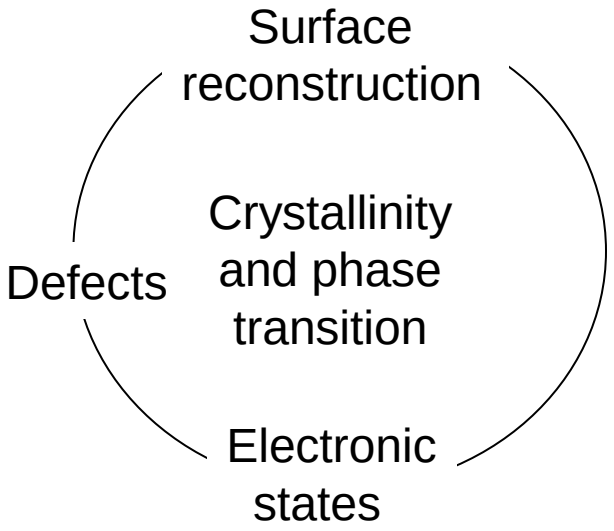
Zhaoxia Ji and Jeffrey Zink

Property Variations in Combinatorial Libraries



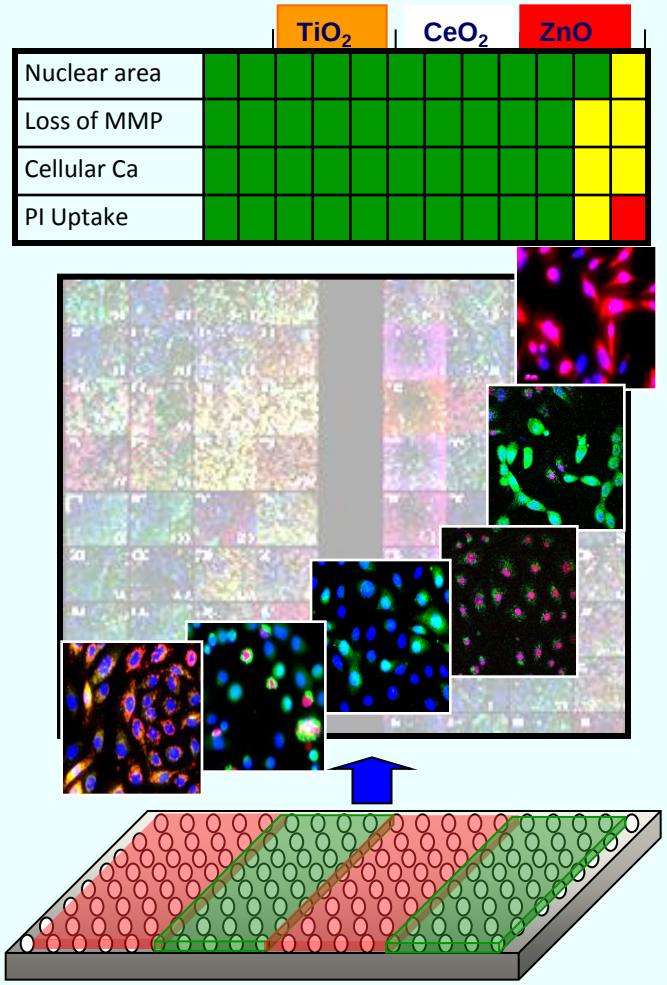






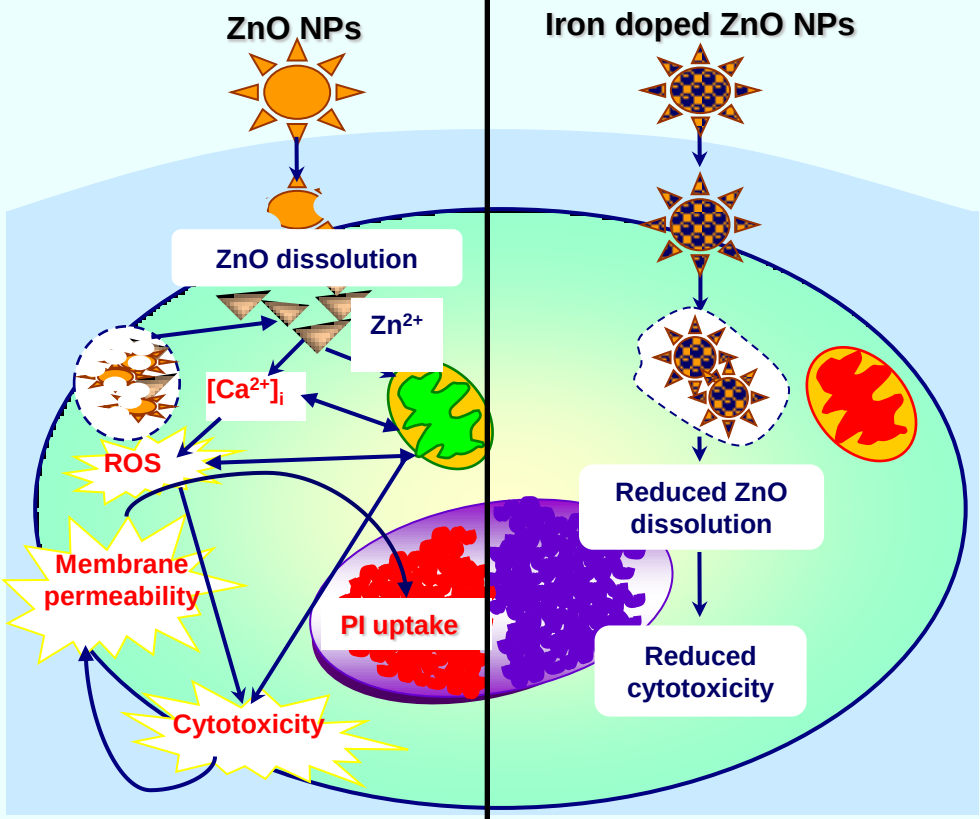
Design of an Fe-doped ZnO library

High throughput toxicity screening



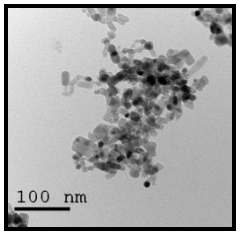
Mechanism of ZnO nanoparticle toxicity

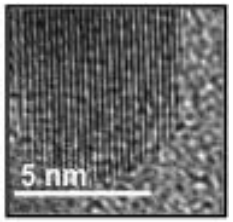
George, S, et al ACS Nano, 2010, 4 (1), pp 15-29.

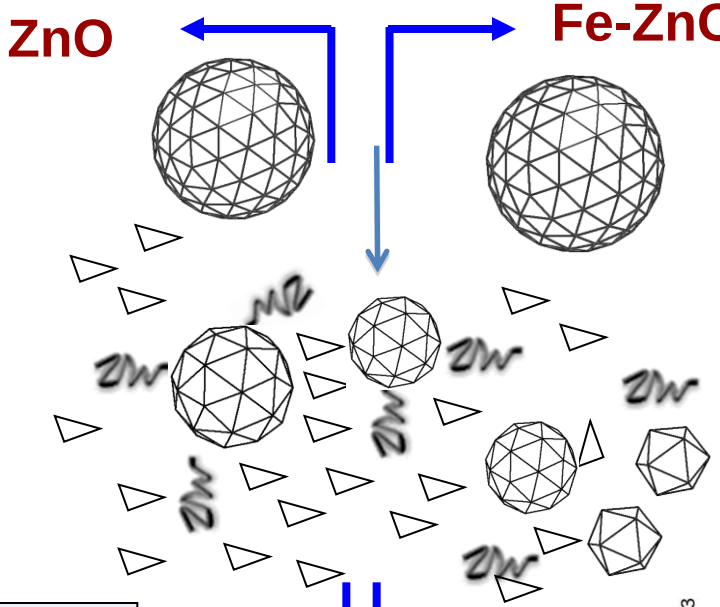


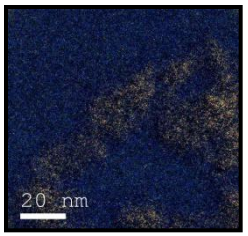
Proposed safe material design

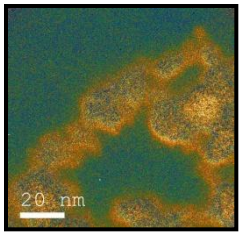
ZnO-based Composition Library to Study the Role of Dissolution Chemistry in Toxicity





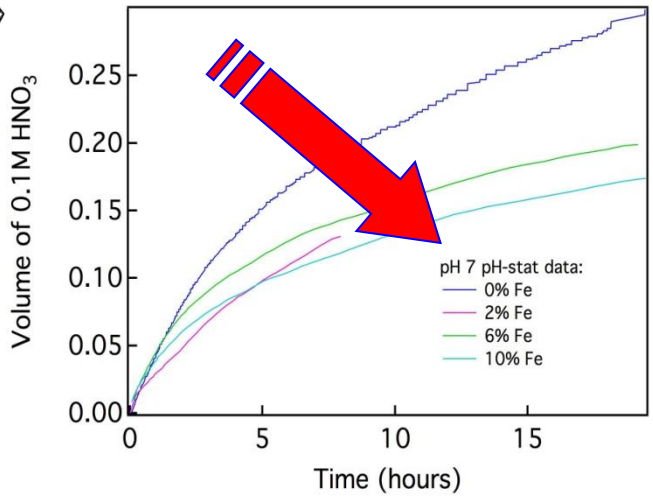






Flame synthesis

Decreased solubility in cell media

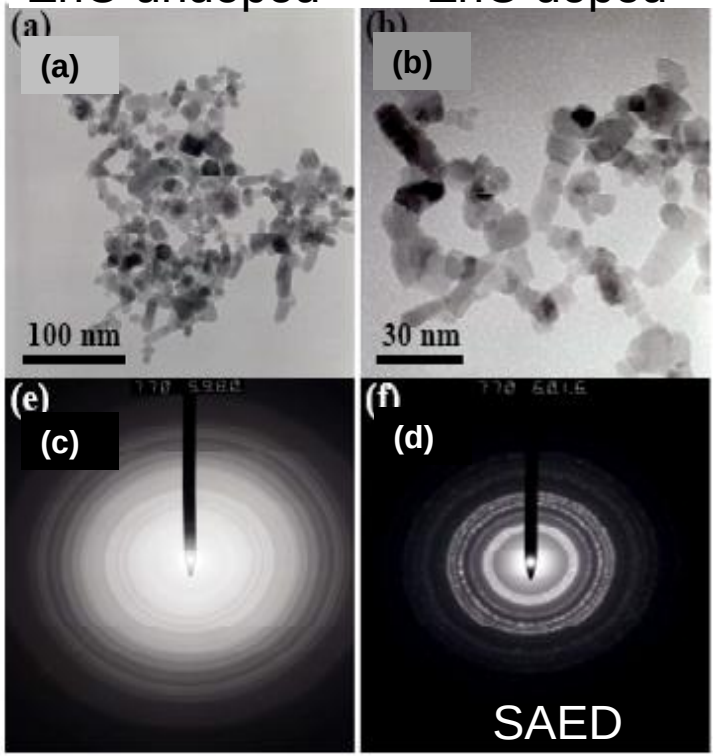


Cellular, bacterial, organism, animal toxicity screening, fate and transport

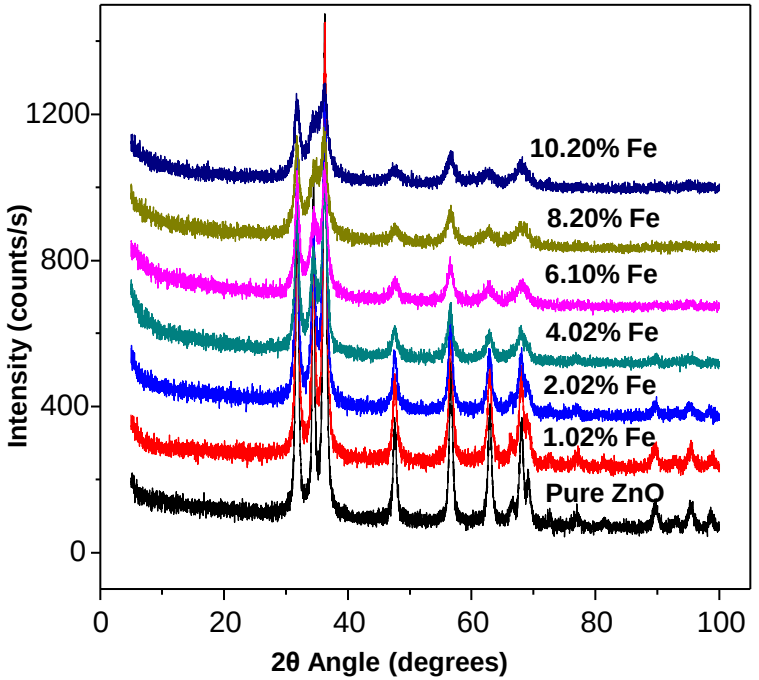
Lutz Mädler, Suman Pokhrel, Bremen

Iron doping alters the matrix and yields slower dissolving ZnO NP

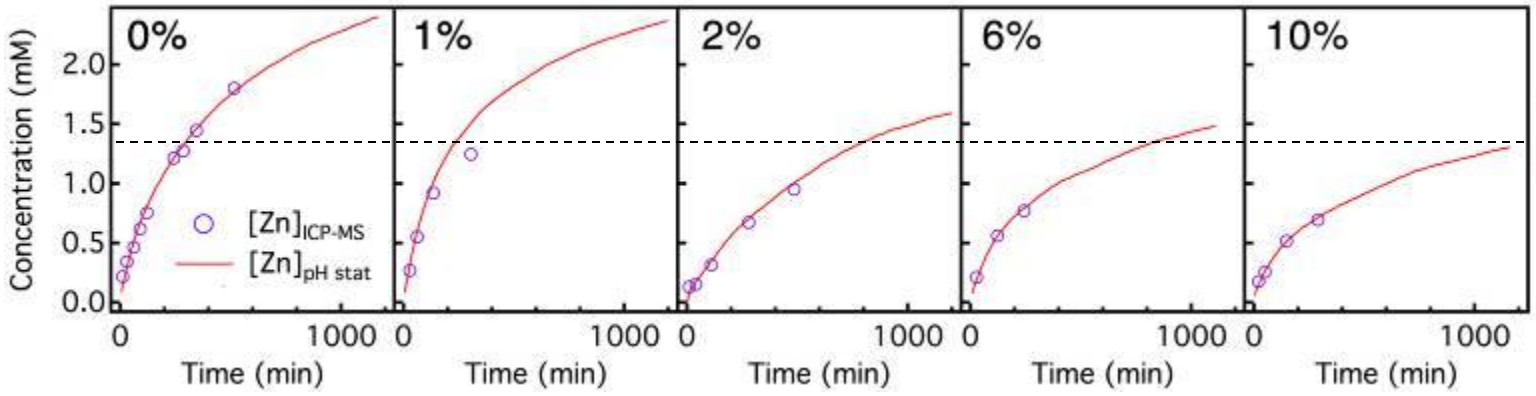
ZnO undoped ZnO doped



Particles synthesized by Lutz Maedler, Germany



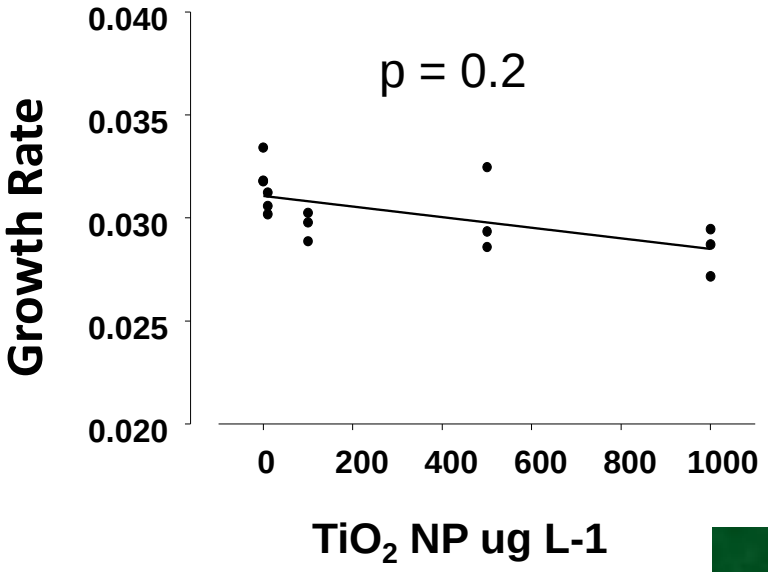
Dissolution rate

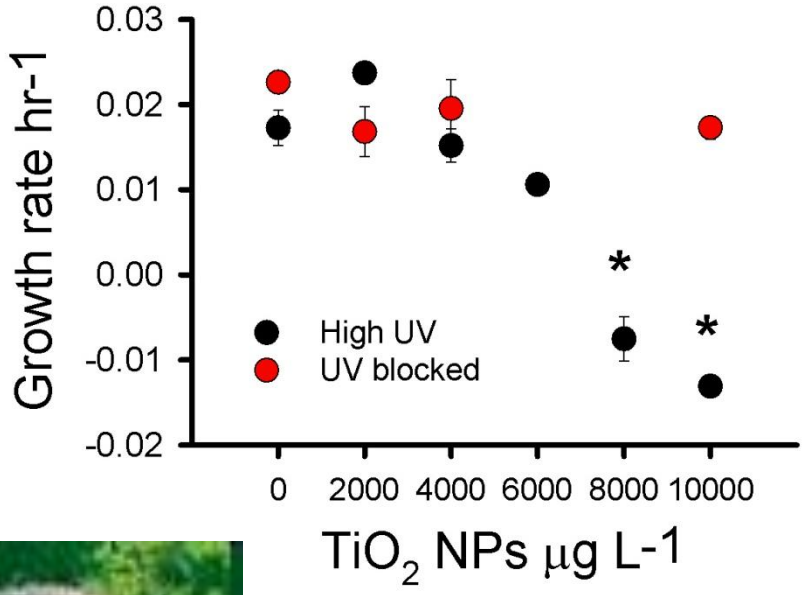


George, Xia, Nel, et al. ACS Nano, 2010, 4, 15–29

NSF: EF-0830117

Low TiO_2 toxicity in Marine Phytoplankton under non-UV conditions changes under UV conditions

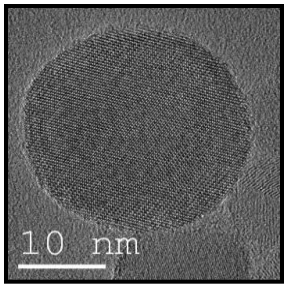




Thalassiosira pseudonana

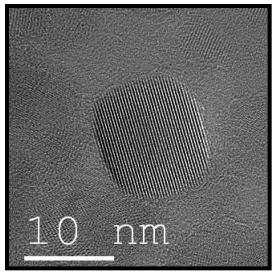
IRG 3-1: Lenihan & Miller

To study photoactivation by TiO_2 mechanistically it is necessary to develop an ENM library that can be used under longer wavelenght conditions: bandgap tuning by Fe doping

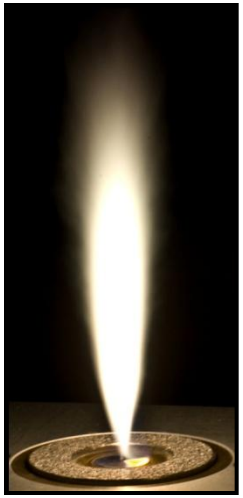


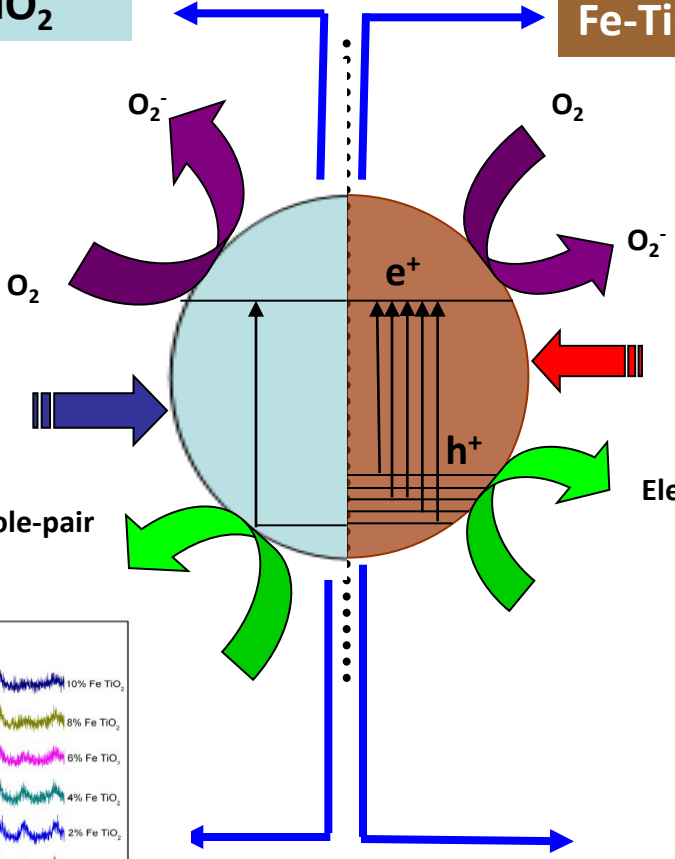
TiO_2

$Fe\text{-}TiO_2$



Flame synthesis





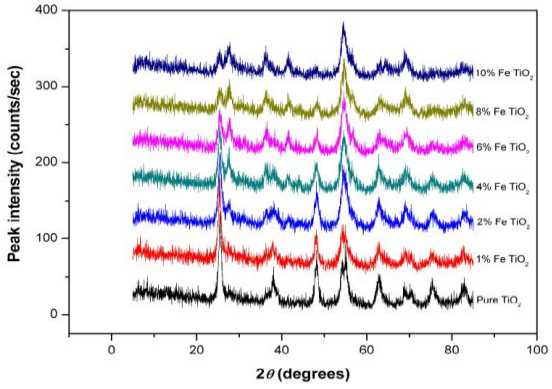
Activation with UV light only

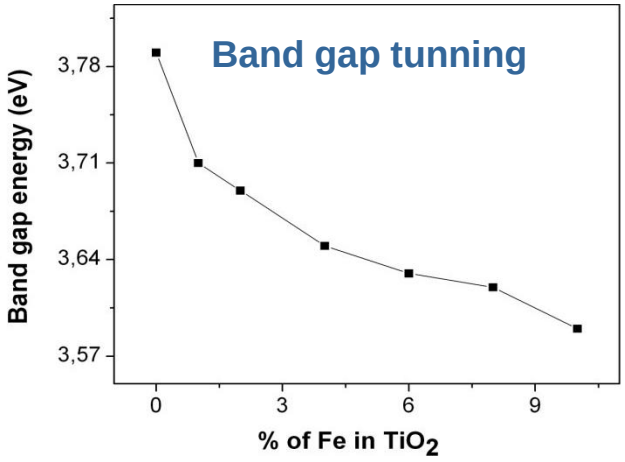
Activation with Visible light

Electron hole-pair

Electron hole-pair

X-ray diffraction

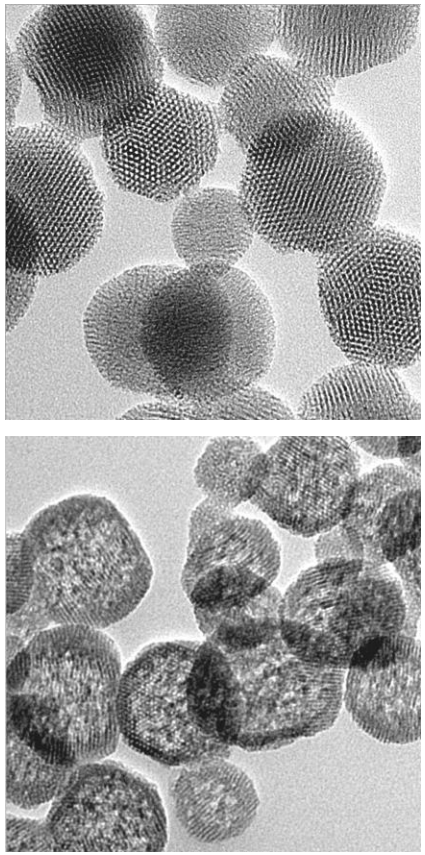




Lutz Mädler, Suman Pokhrel, Bremen

UCLA NanoMachine Center

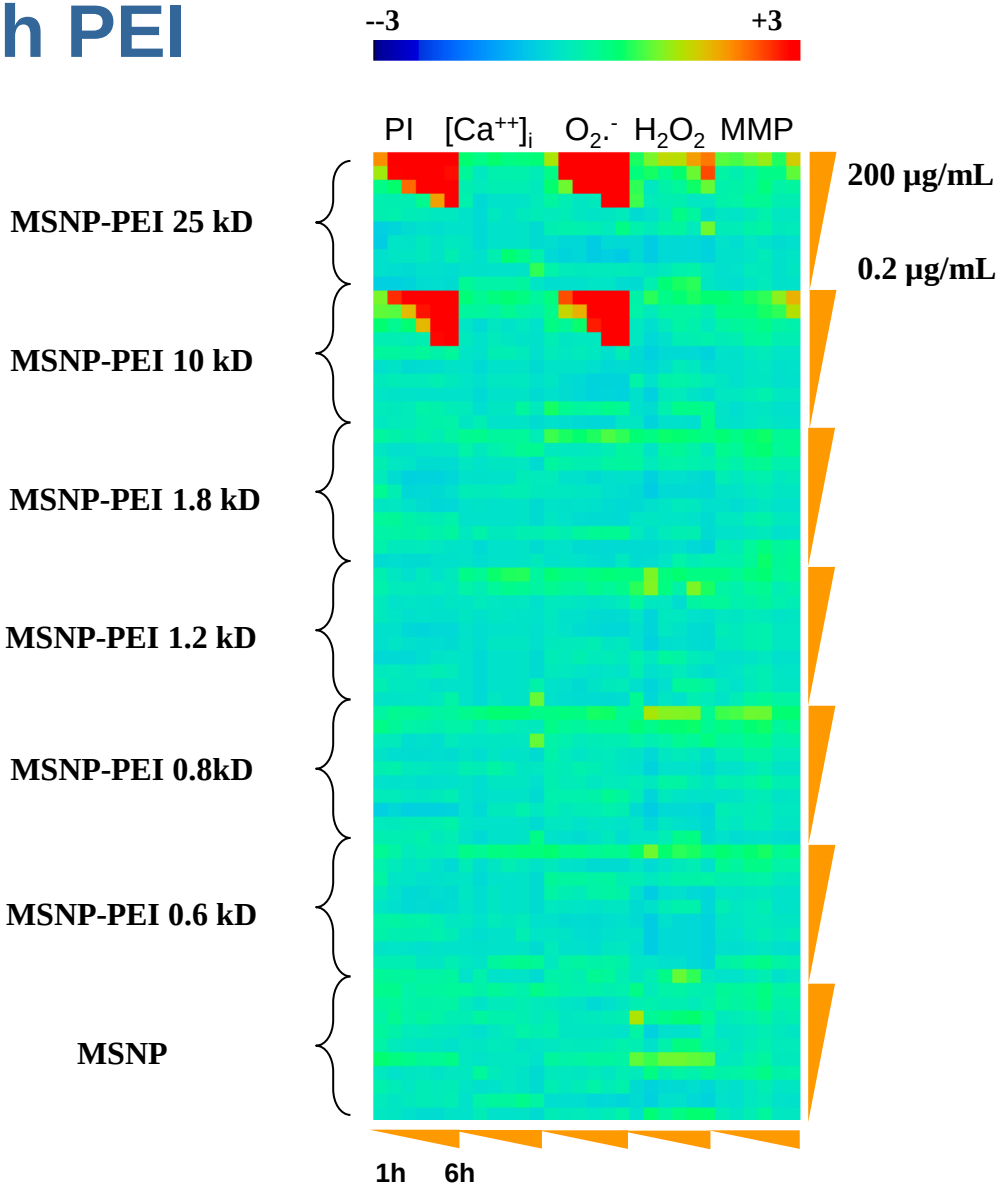
Construction of a cationic MSNP library by coating with PEI



(PEI)

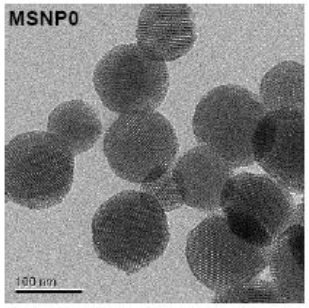
0.6 kD
0.8 kD
1.2 kD
1.8 kD
10 kD
25 kD

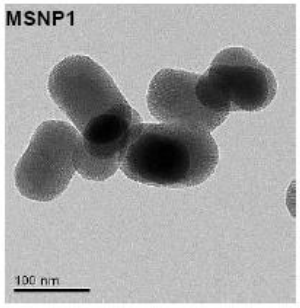
Cancer cell lines
NHBE

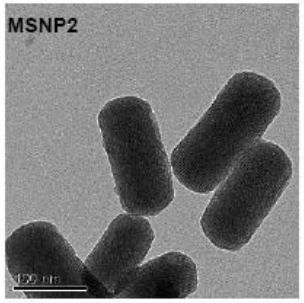


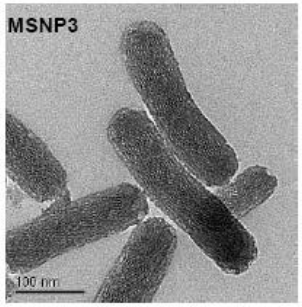
Xia et al. ACS Nano. 2009

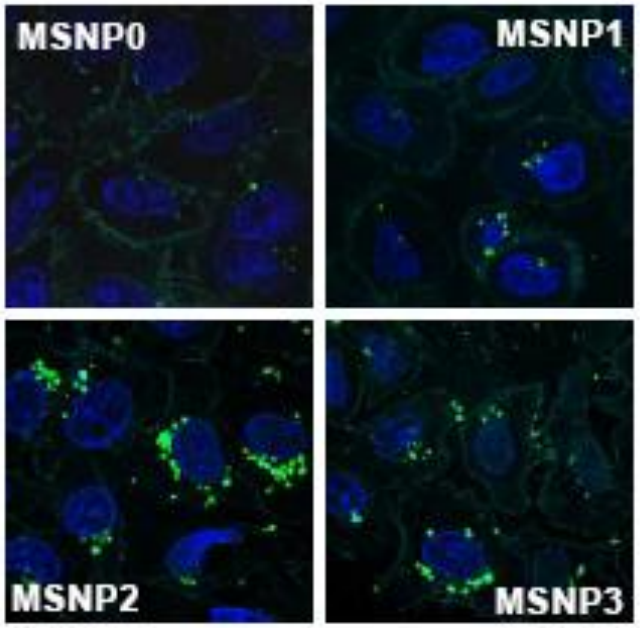
Shape and Aspect Ratio Property Library shows that aspect ratio has a profound effect in active uptake tied to a specific Cellar activation mechanism

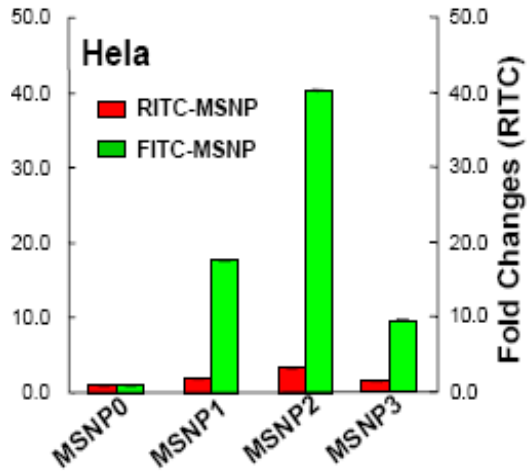


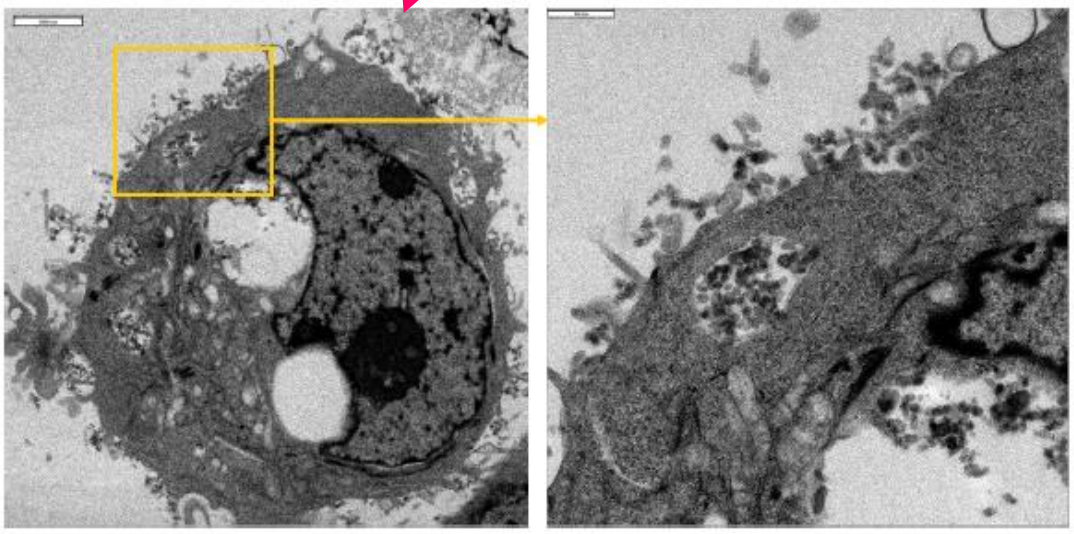












What linkages can be used for high content data generation to prioritize *in vivo* assessment?

Bio-Molecules
- Genes
- Proteins, etc

Cellular injury responses
- Non-lethal
- Lethal

Biological pathways
- Toxicological
- Signaling
- Death pathways

Single cell or simpler life forms

Animal pathology, disease
- Embryos
- Vertebrates
- Mammalian

Human health impact

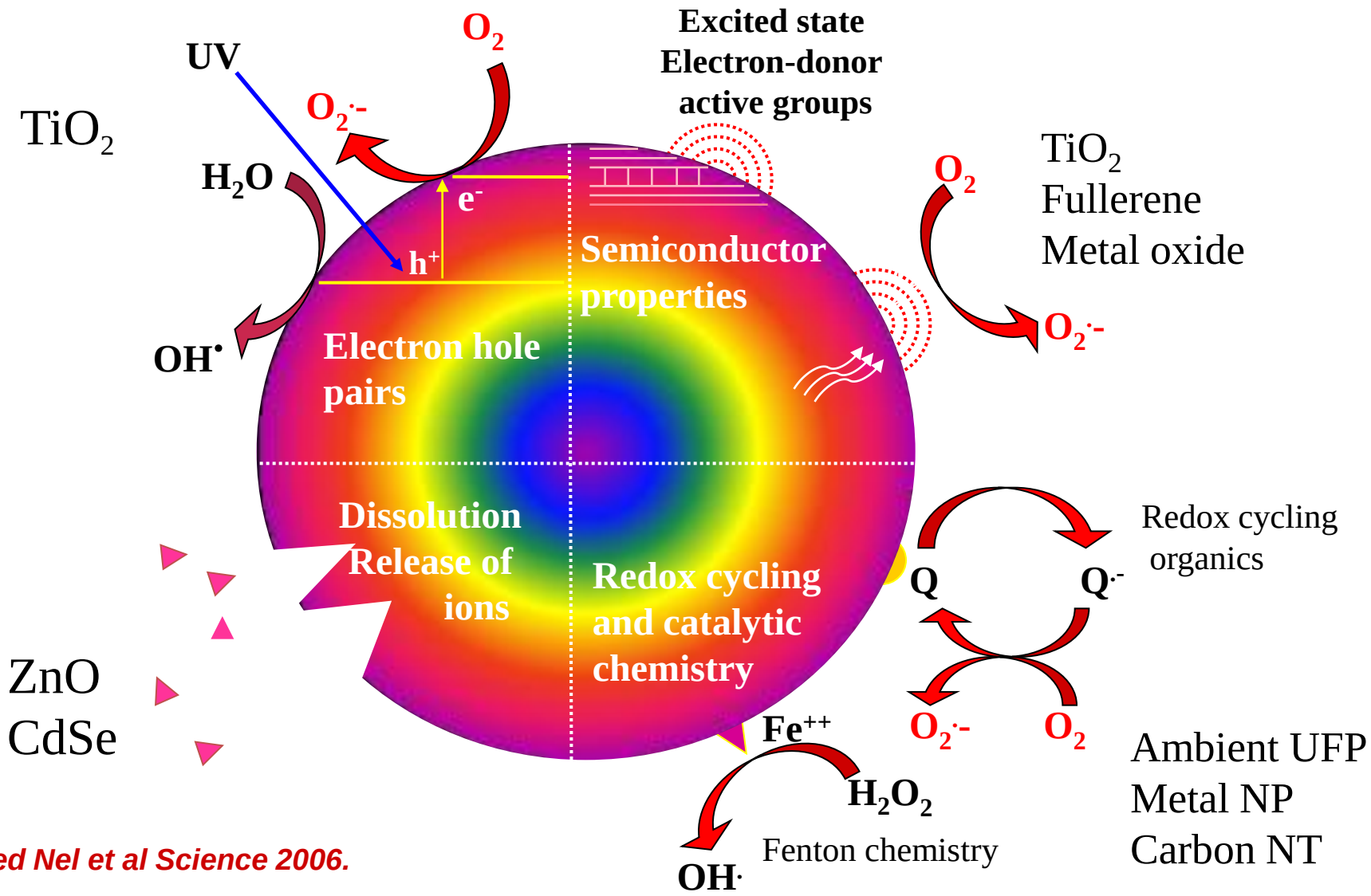
Impact on higher life forms, predators, populations and ecosystems

Robust Screening Platform: Mechanistic Cellular Injury Pathways

Toxicological Pathway	Example Nanomaterials
Membrane damage/leakage	Cationic NPs
DNA cleavage/mutation	Nano-Ag
Mitochondrial damage & apoptosis	ZnO, cationic NPs
Lysosomal damage: proton sponge effect frustrated phagocytosis	Cationic NPs CNTs
Fibrogenesis and tissue remodeling	CNTs
Blood platelet, vascular endothelial &clotting abnormalities	SiO_2
Signaling cascades Inflammation, gene expression, survival	Metal oxide NPs, CNTs
Oxidative stress injury	CNTs, metal oxide NPs, cationic NPs

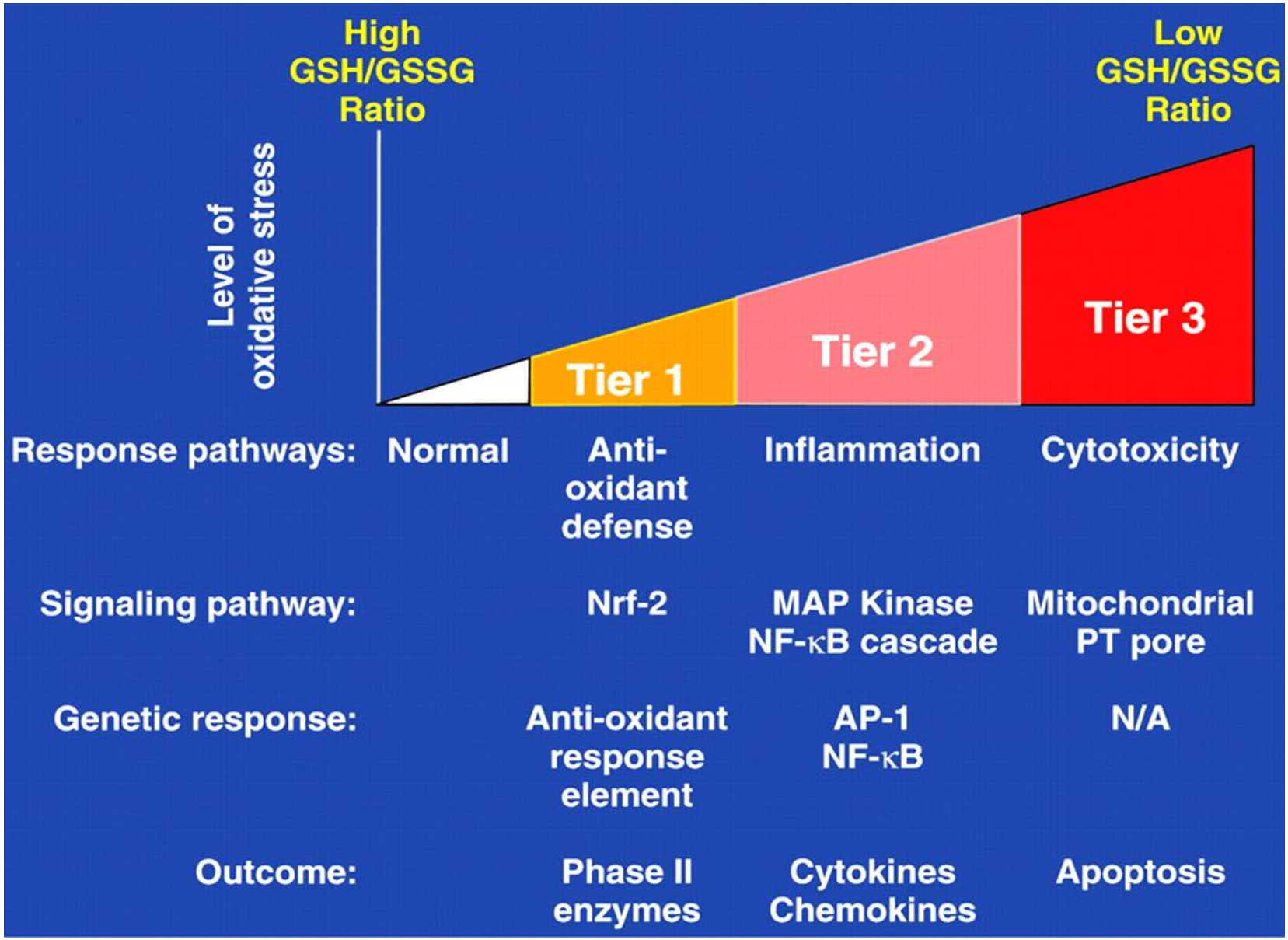
Huan Meng et al ACS Nano. 2009

Nanomaterial Mechanisms for Oxygen Radical production



Adapted Nel et al Science 2006.

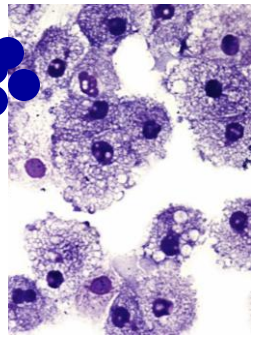
The Hierarchical Oxidative Stress Model



Nel et al. Science, 311, 622-627, 2006

In vitro comparison of a panel of nanoparticles based on the hierarchical oxidative stress paradigm

Epithelial
Endothelial
Macrophage
Kidney
Liver
Neuronal
etc



Abiotic
Biotic

Signaling
JNK
NF-κB

$[Ca^{2+}]_i$

Tier 1
Phase 2 anti-ox enzymes
HO-1
GSH

Tier 2
Inflammation
cytokines
chemokines

Tier 3
Mitochondria
MMP
ATP
ROS
$[Ca^{2+}]_m$

Cell death
caspase activation
PI uptake
MTS assay

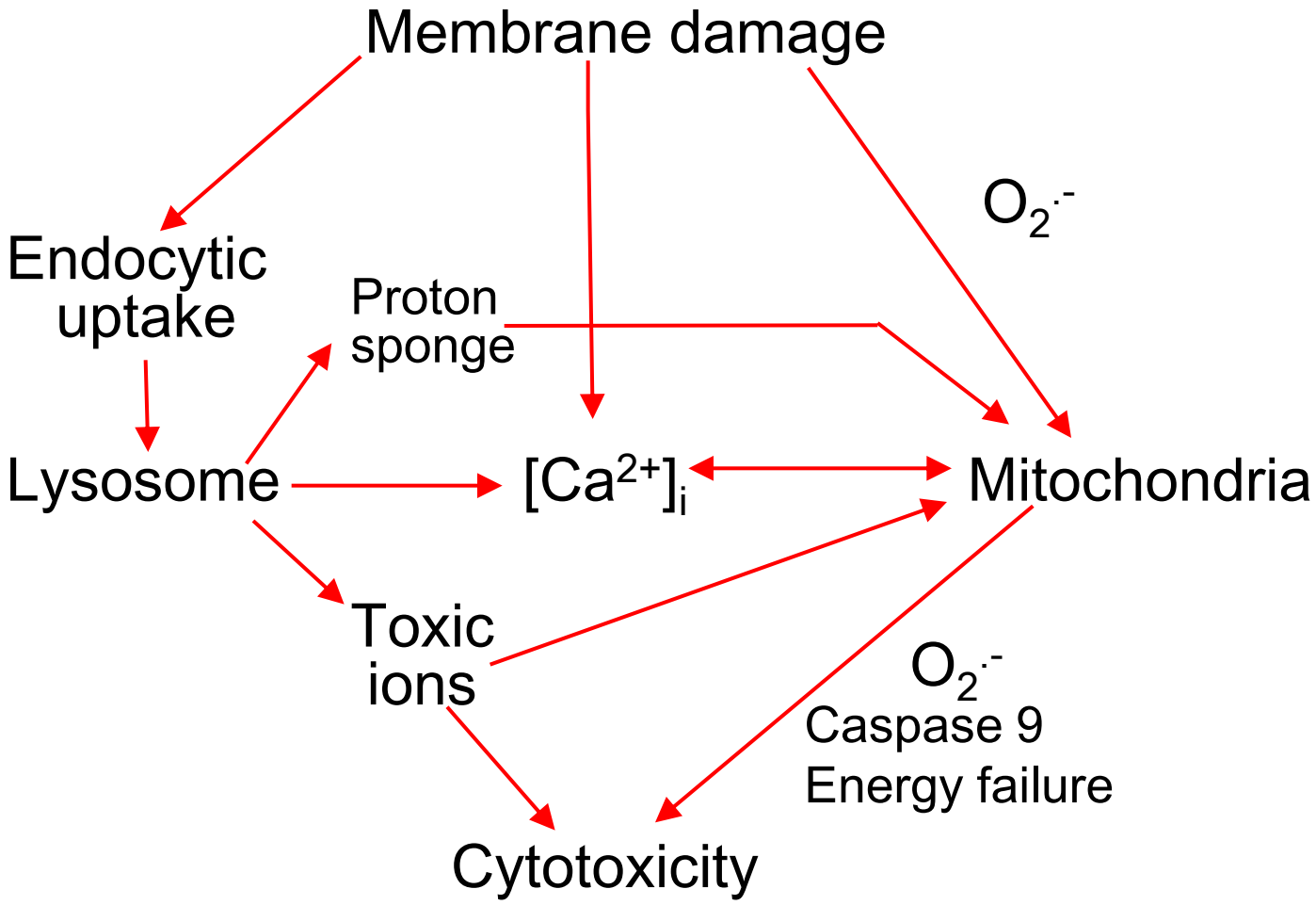
CB
Polystyrene
TiO_2
Fullerol
UFP
NH2-Ps
etc

It is possible to do profiling:

	ROS	Tier 1	Tier 2	Tier 3
Particle 1				
Particle 2	particle			
Particle 3	particle			
Particle 4	particle			
Particle 5	particle			
Particle 6	cell			

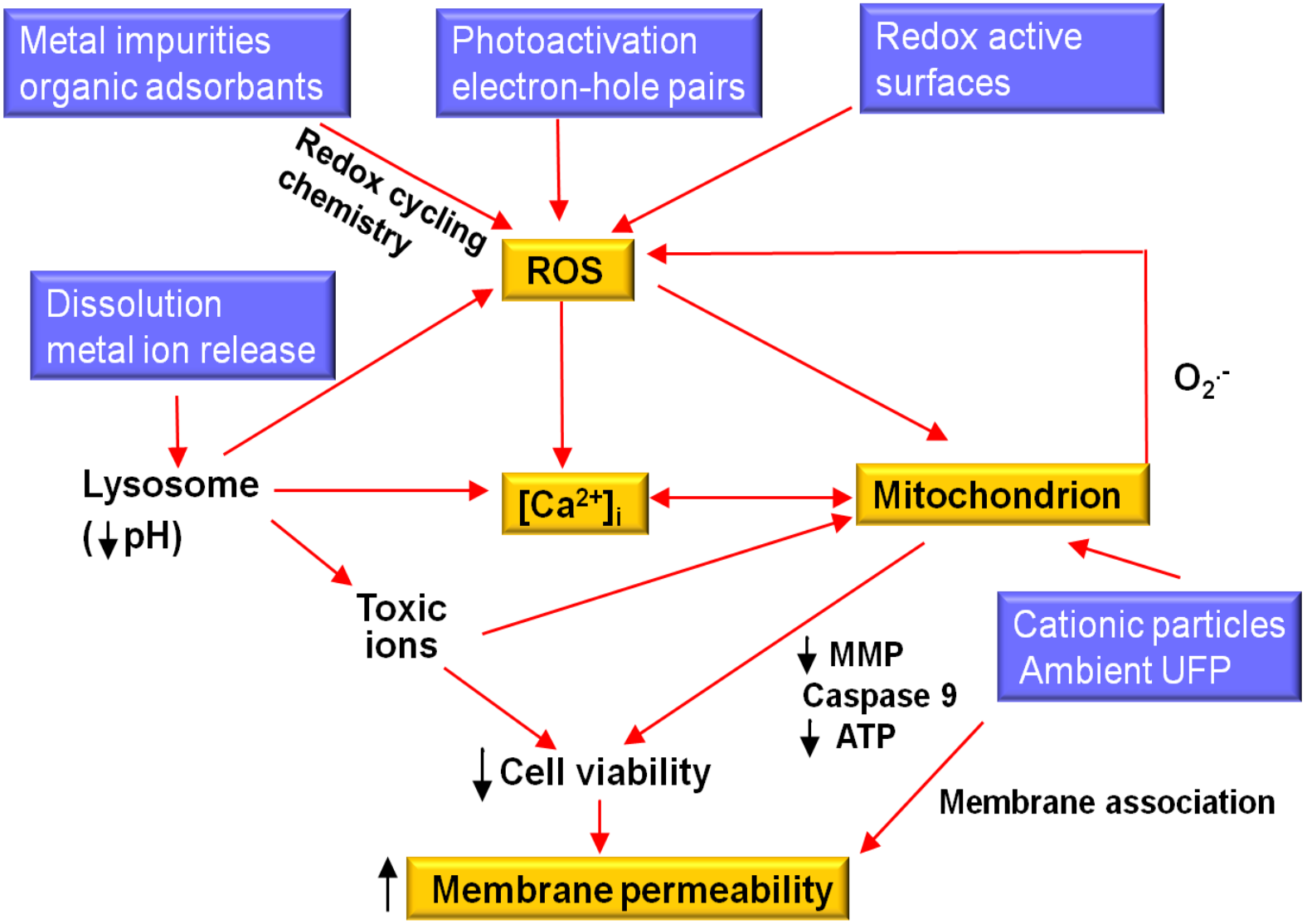
Xia et al. Nano Letters. 2006
Meng H et al. ACS Nano 2009

Interconnected final common pathways of NM injury to screen for lethal and sublethal responses



George et al. ACS Nano. 2010

Establishment of a Multi-parametric Assay

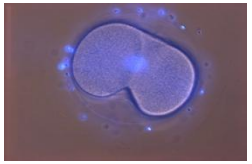


George et al. ACS Nano. 2010

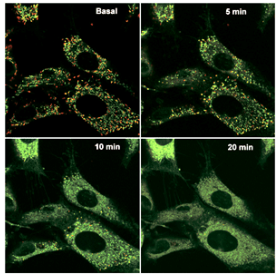
Multi-parametric Oxidative stress High Throughput Screen

- ROS generation
- Mitochondrial membrane depolarization
- Cytotoxicity and PI uptake
- Intracellular Ca flux
- Cell localization / nucleus

Cells
Bacteria
Yeasts
Embryos



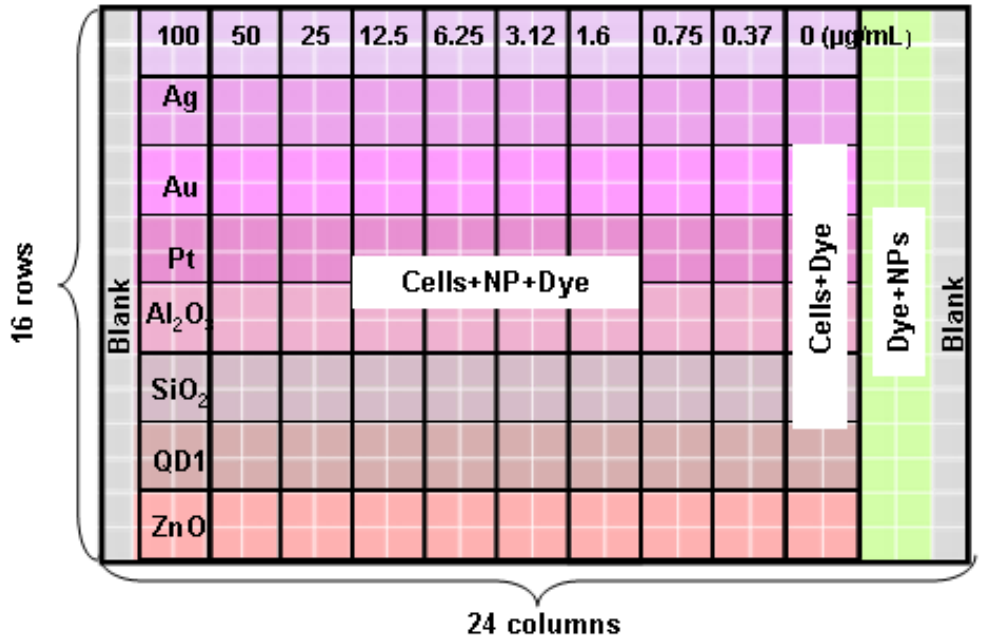
ENM



Epi-fluorescence microscopy

Group Leader: ***Ken Bradley (UCLA)***
MSSR Director: ***Robert Damoiseaux***

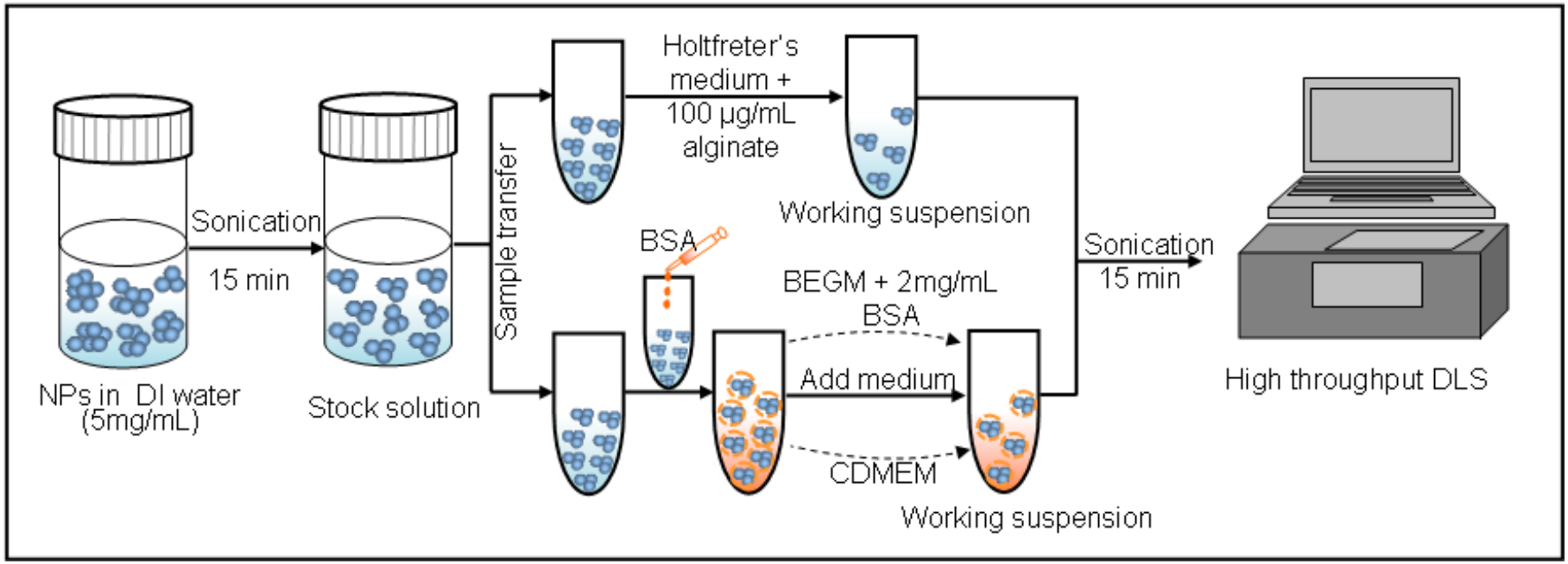
Plate layout for each cell type



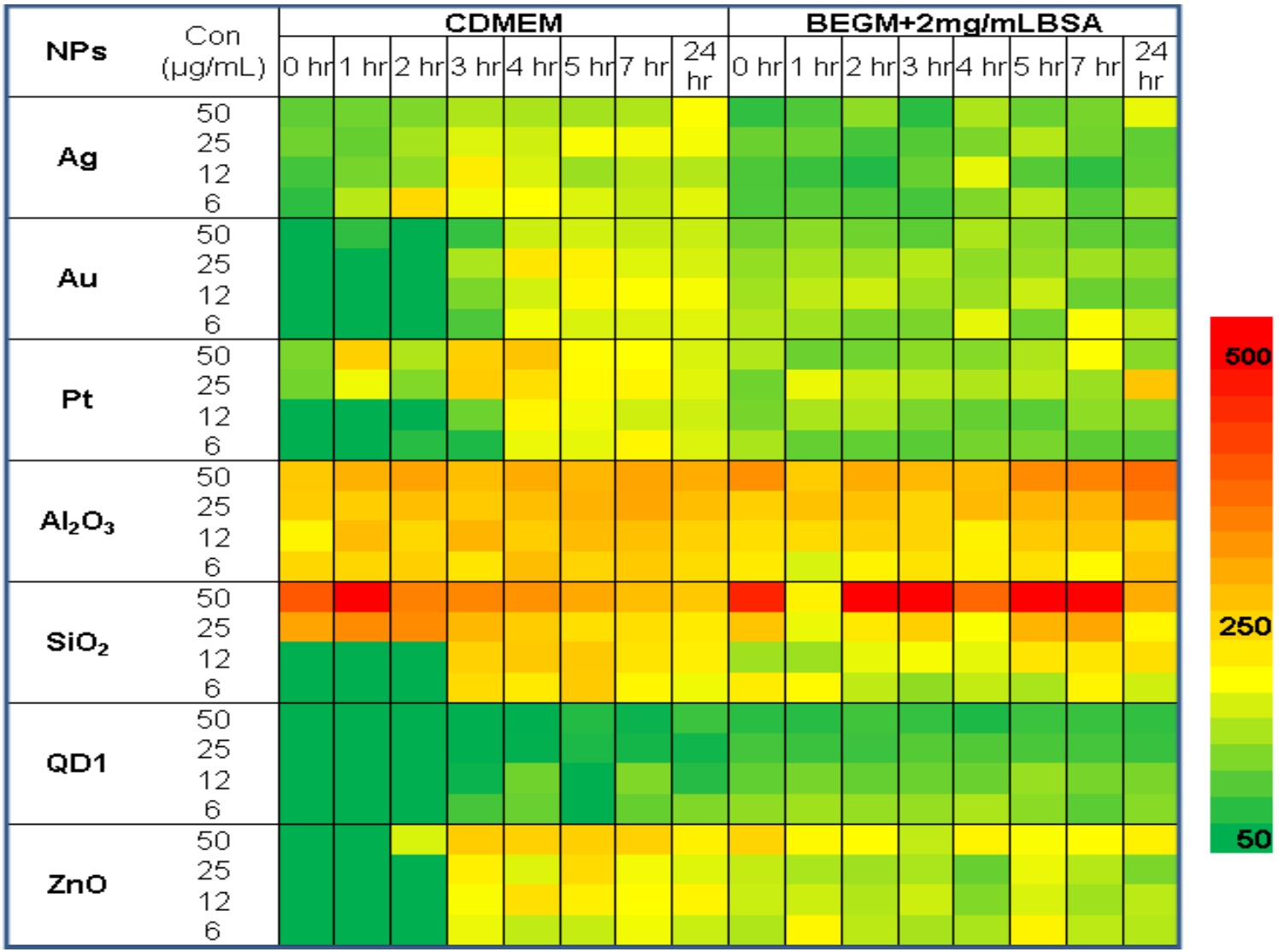
HTS of a Metal and Metal compositional series

NMs	Size (nm)			Zeta potential (mV)		
	Water	CDMEM	BEGM+BSA (2mg/mL)	Water	CDMEM	BEGM+BSA (2mg/mL)
Ag	95.68	77.17	110	-30.7	-10.2	-8.77
Au	294.45	21.9	29.1	-17.4	-6.15	-1.93
Pt	271	28.6	173	-34.1	-9.26	-8.58
Al_2O_3	1168	25.8	57	-8.77	-10.6	-8.47
SiO_2	1135.35	22.6	67.91	-31.6	-6.54	-10.4
Qdot-T	168.5	48.5	443.2	78.4	-10.3	-10.1
ZnO	130.5	24.23	45.17	17.4	-7.16	-7.93

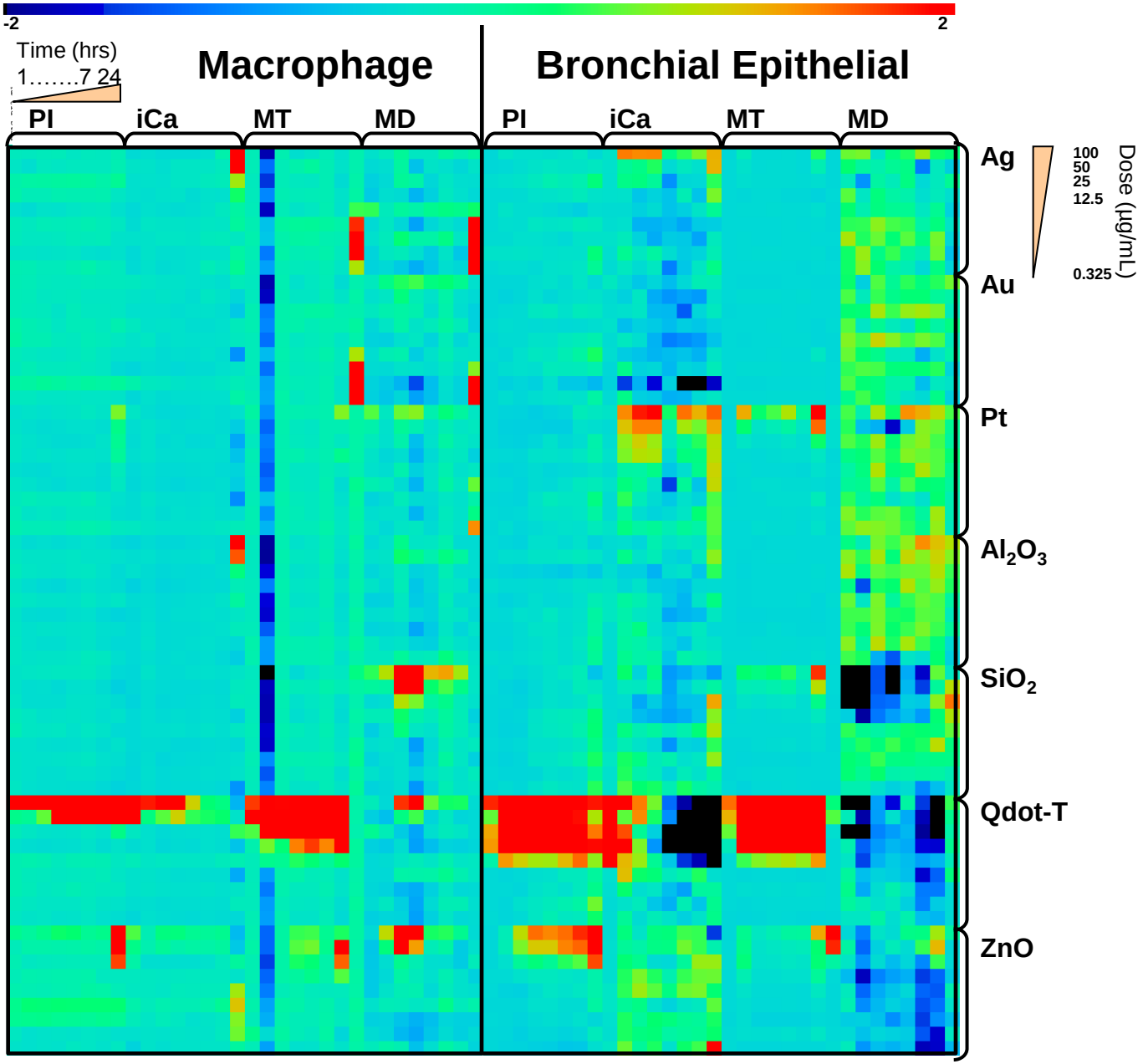
HTS requires high throughput methods to assess particle suspension and stability



High Throughput DLS of the Kinetics of NP agglomeration in mammalian tissue culture media



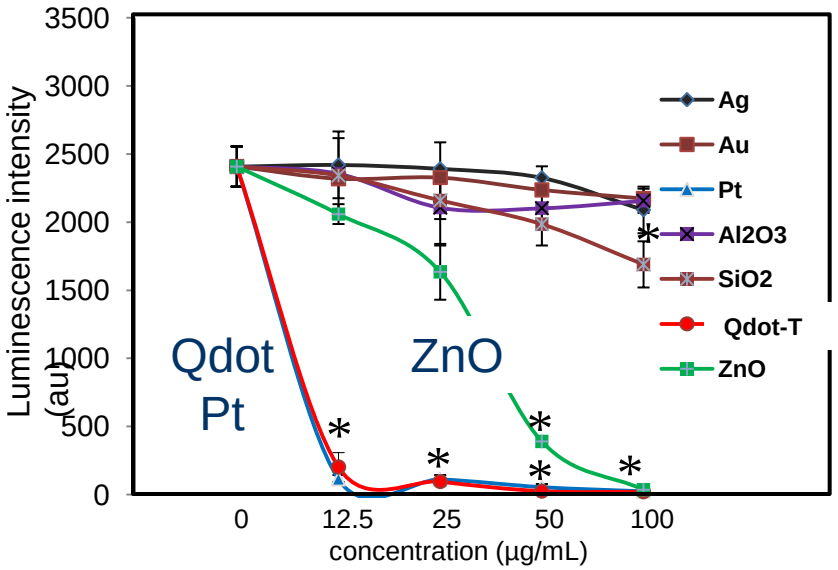
Heat Map of the multi-parametric data (z-score transformation)



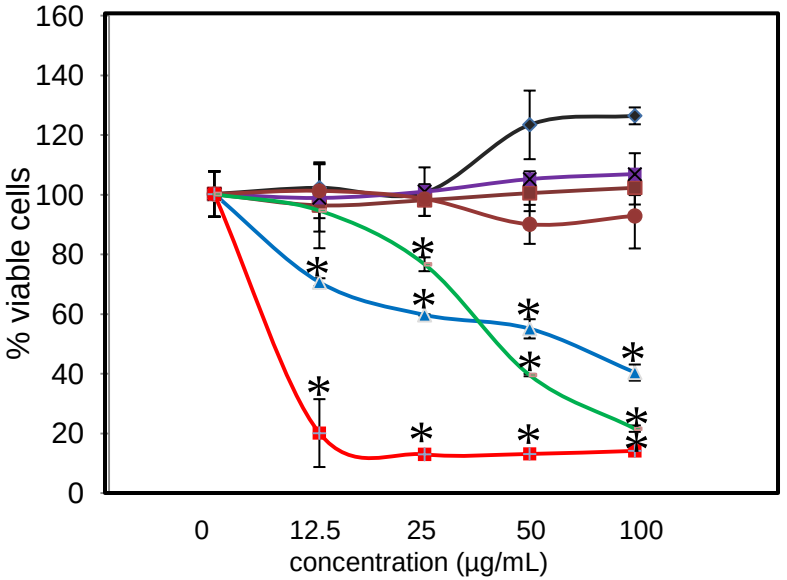
Comparison of multi-parametric data to individual responses assessment

Macrophage

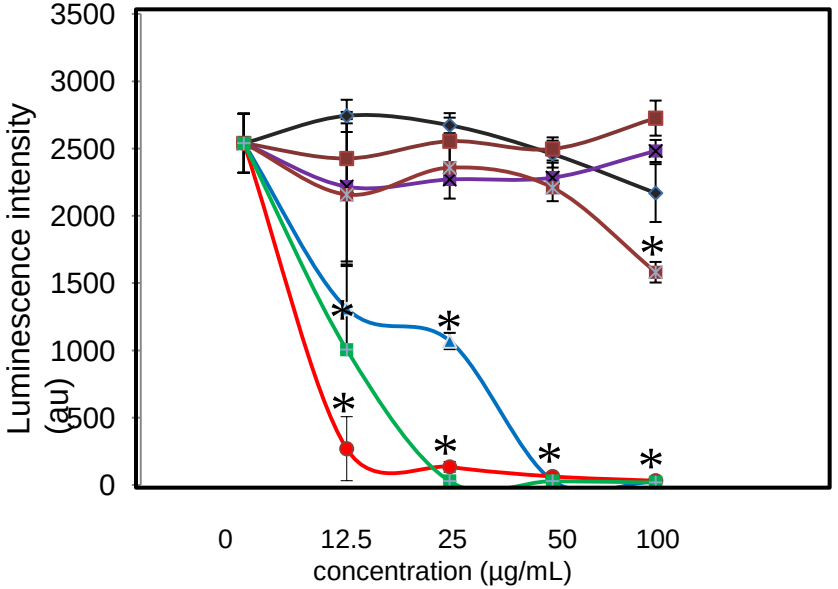
ATP measurement

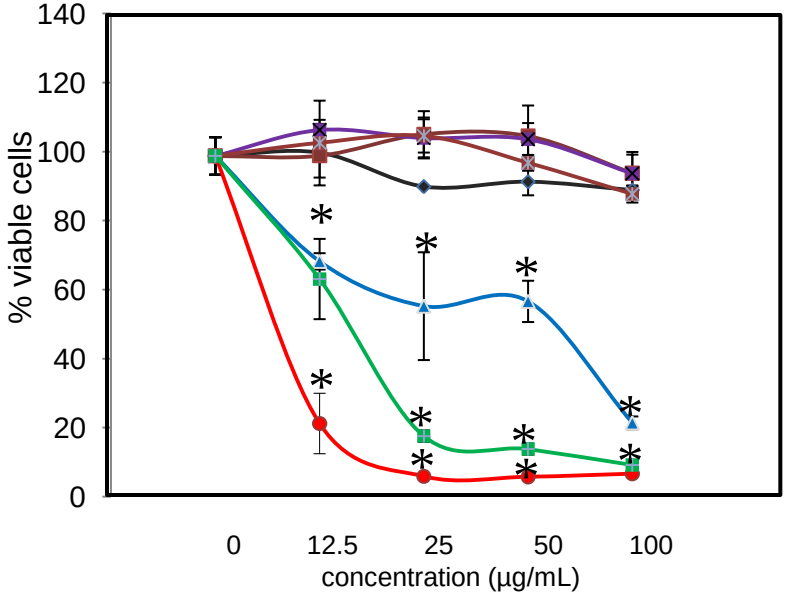


Cell viability by MTS assay

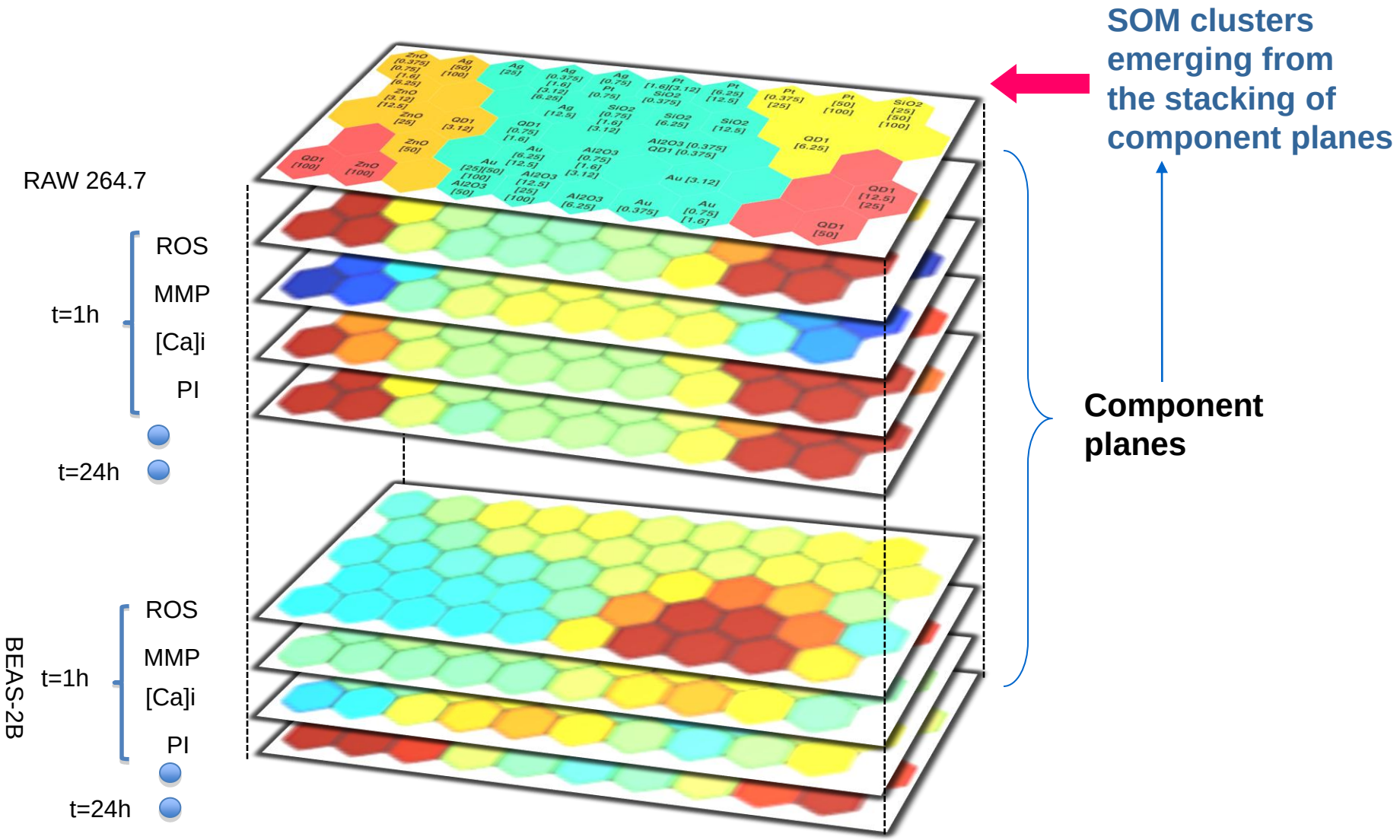


Bronchial Epithelial



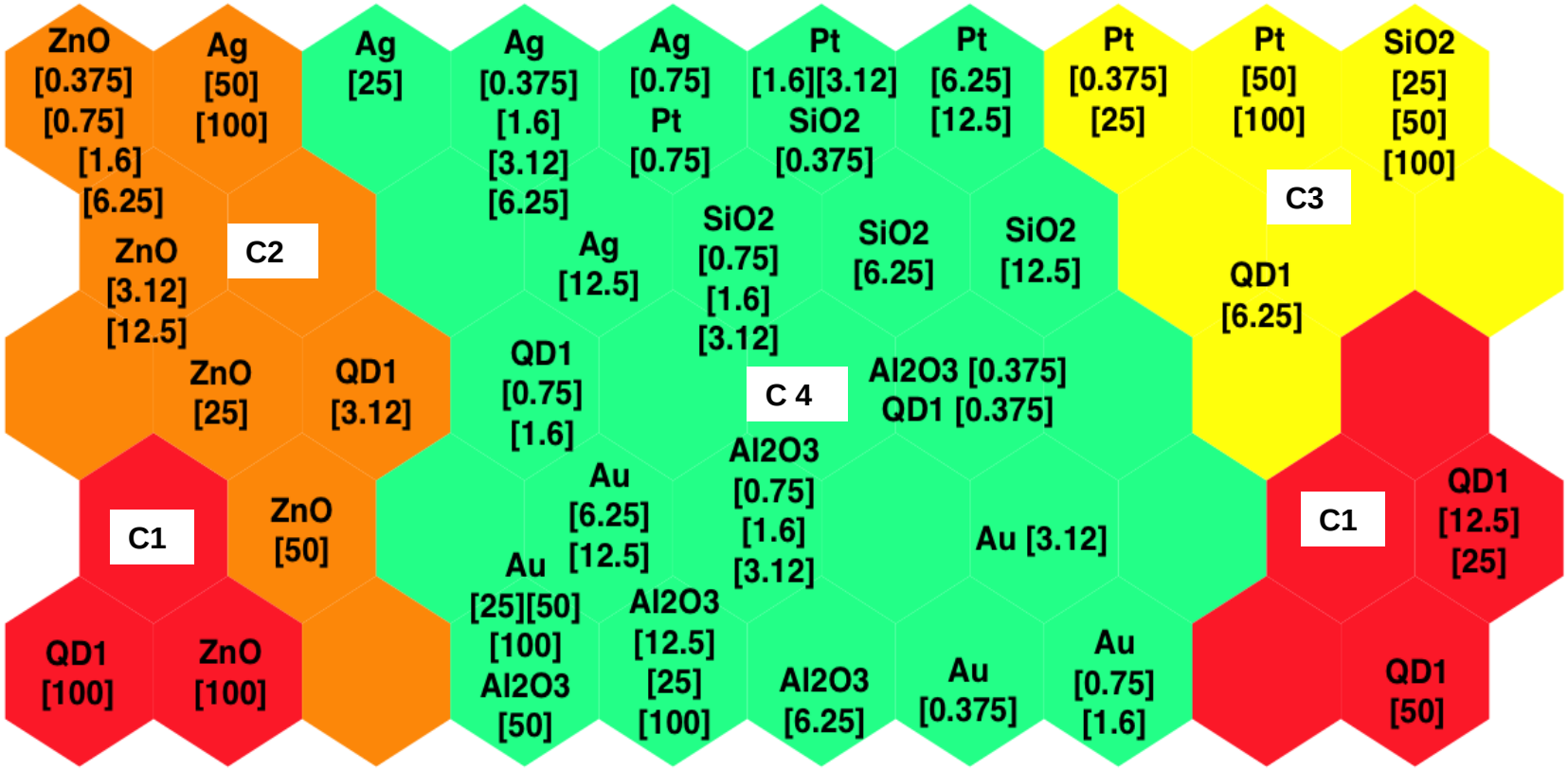


Advantage of Multi-parametric testing: Revealing hidden relationships

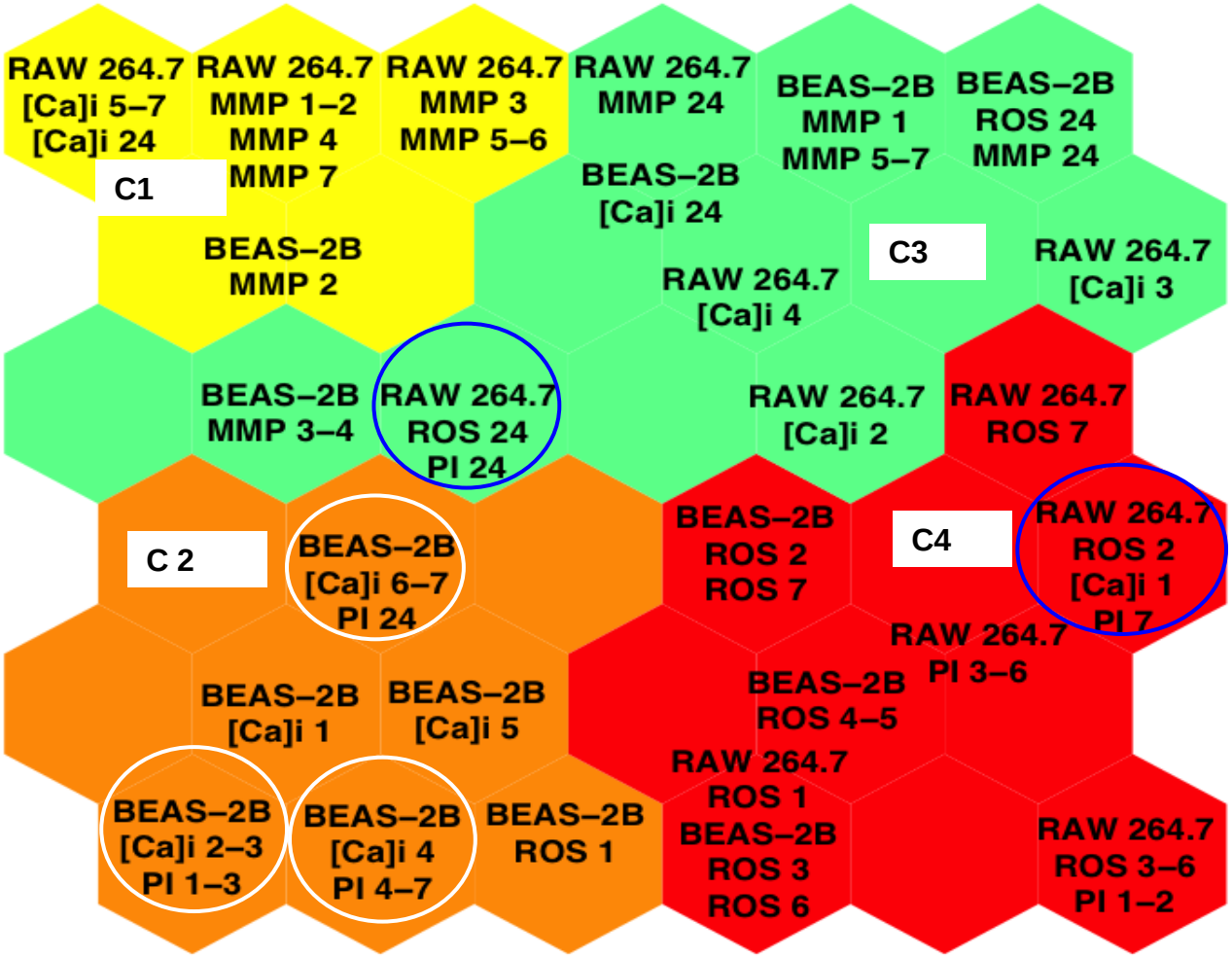


2D visualization of the relationship between all cytotoxic parameters, all doses, time points and for all particles

SOM defined by similarity in cytotoxic response profiling for the entire data set



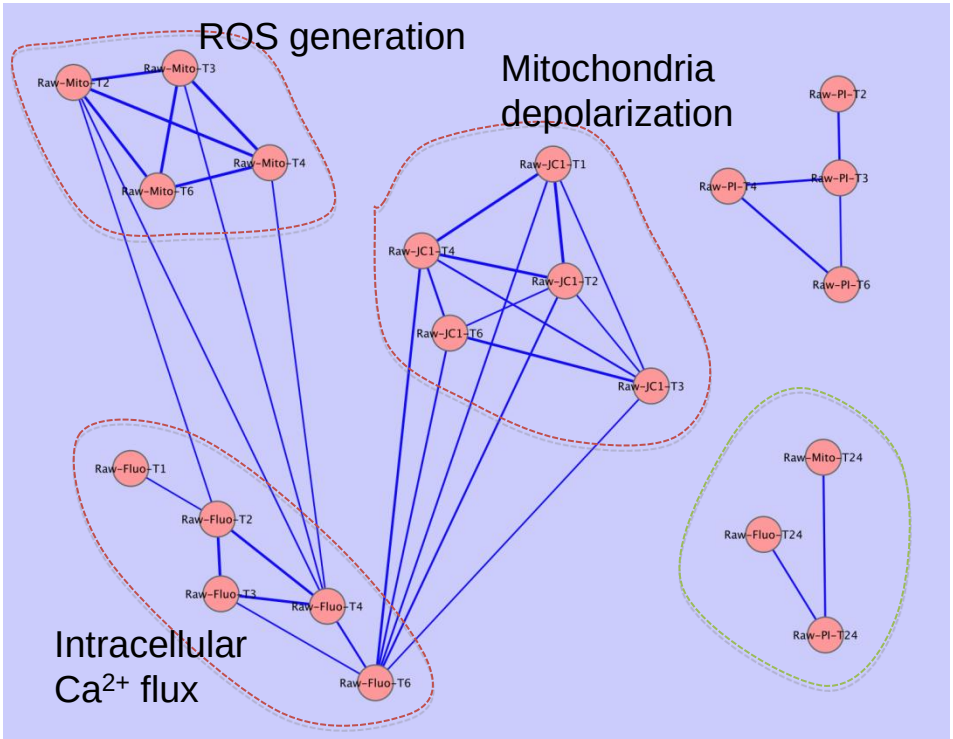
SOM defined by clustering of the biological response characteristics



NSF: EF-0830117

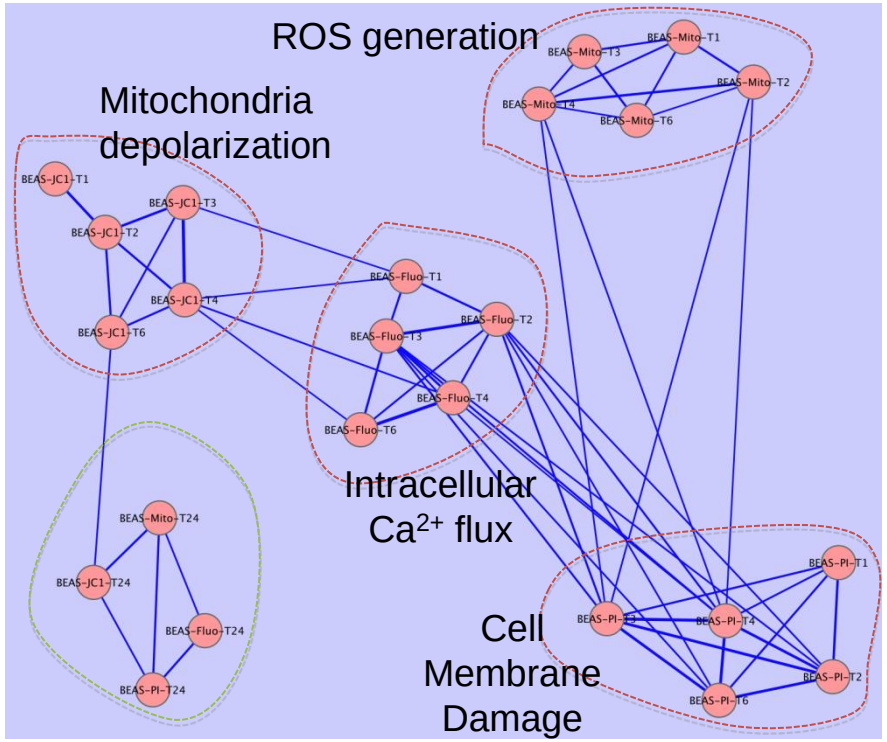
Activity-Activity Relationships

RAW 264.7 cells



Correlation threshold: |r|=0.5

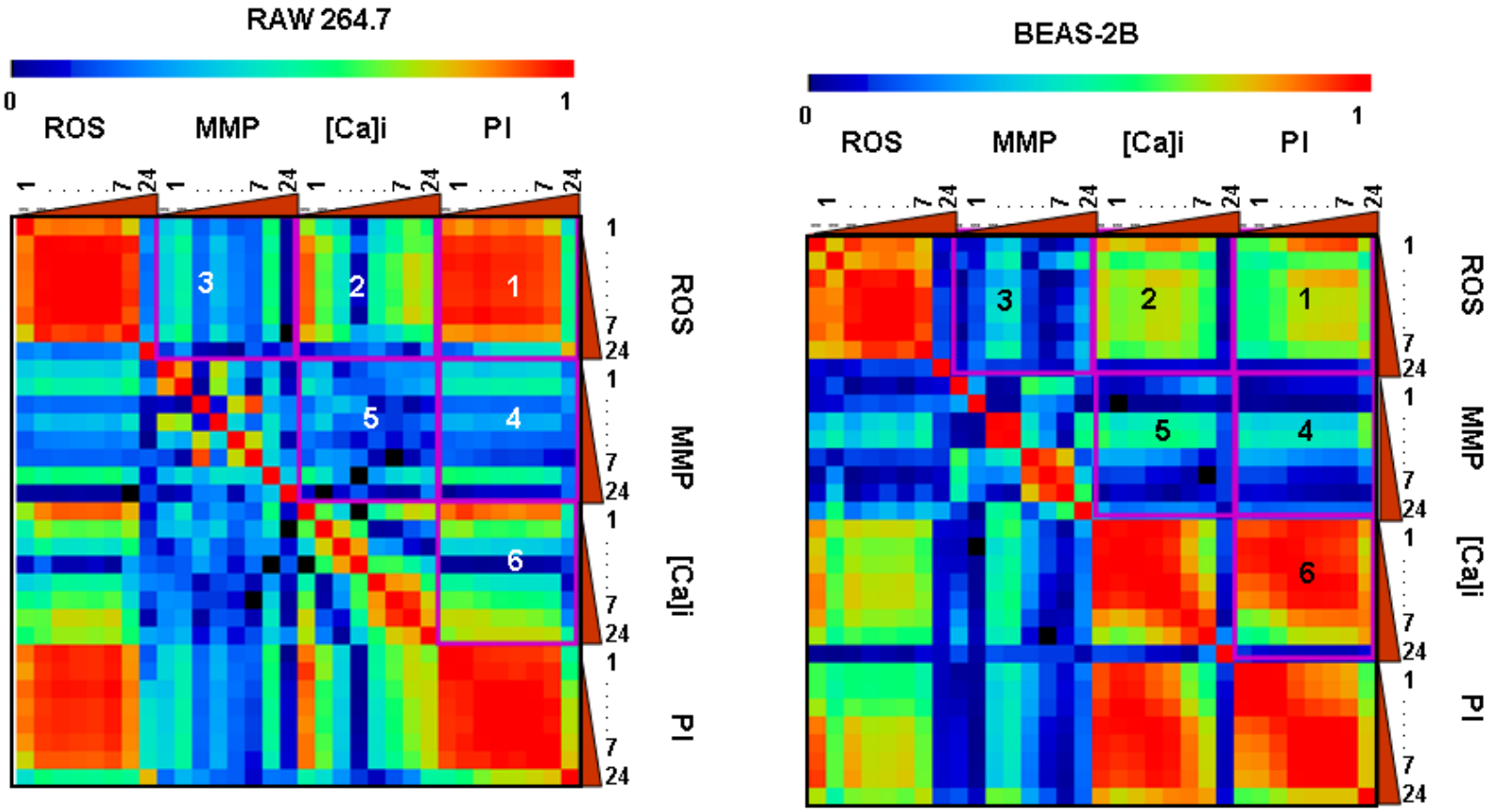
BEAS-2B cells



Effects after long exposure time: 24h

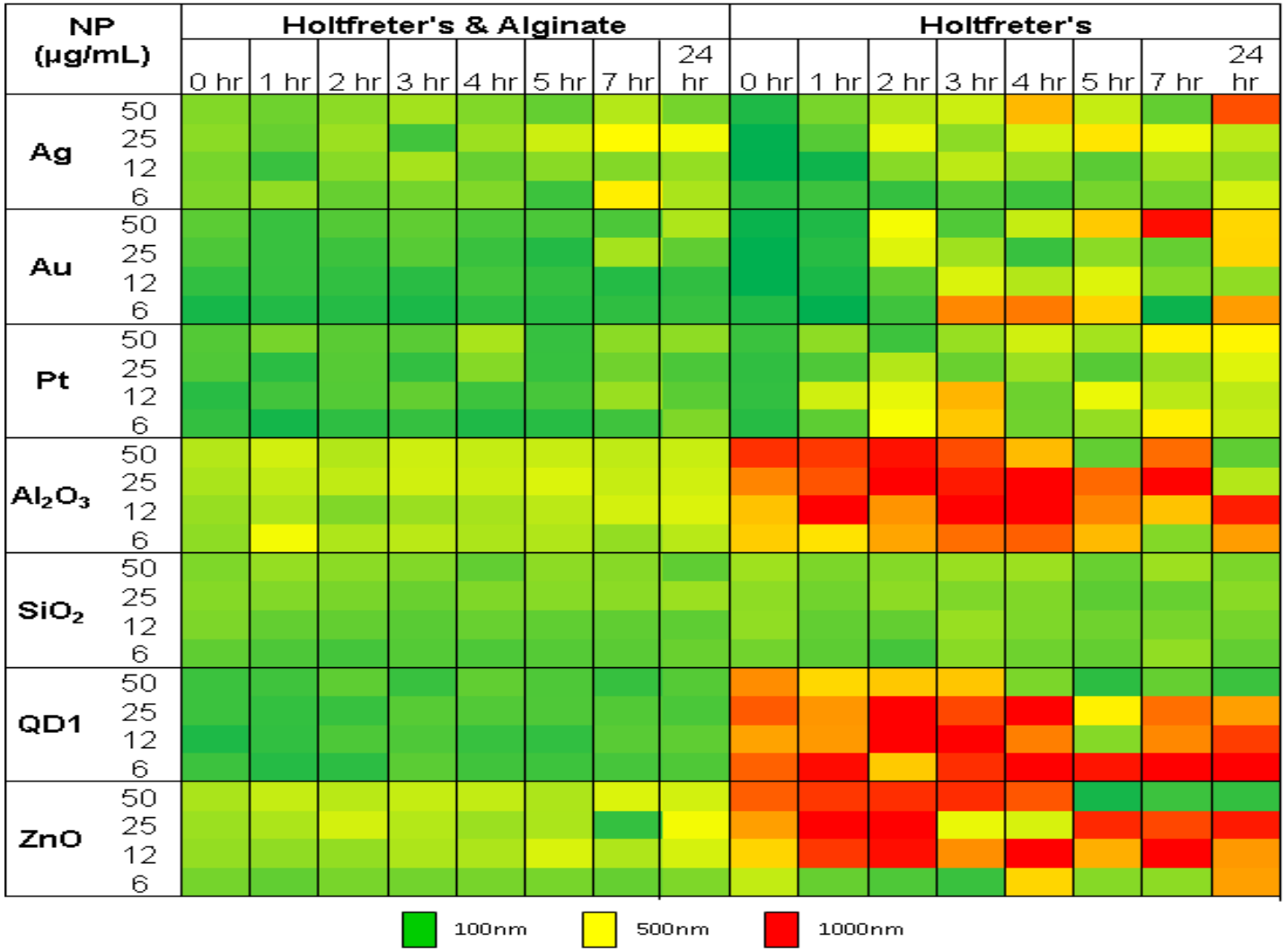
NSF: EF-0830117

Heat map display showing cellular correlation matrixes for each cytotoxicity parameter

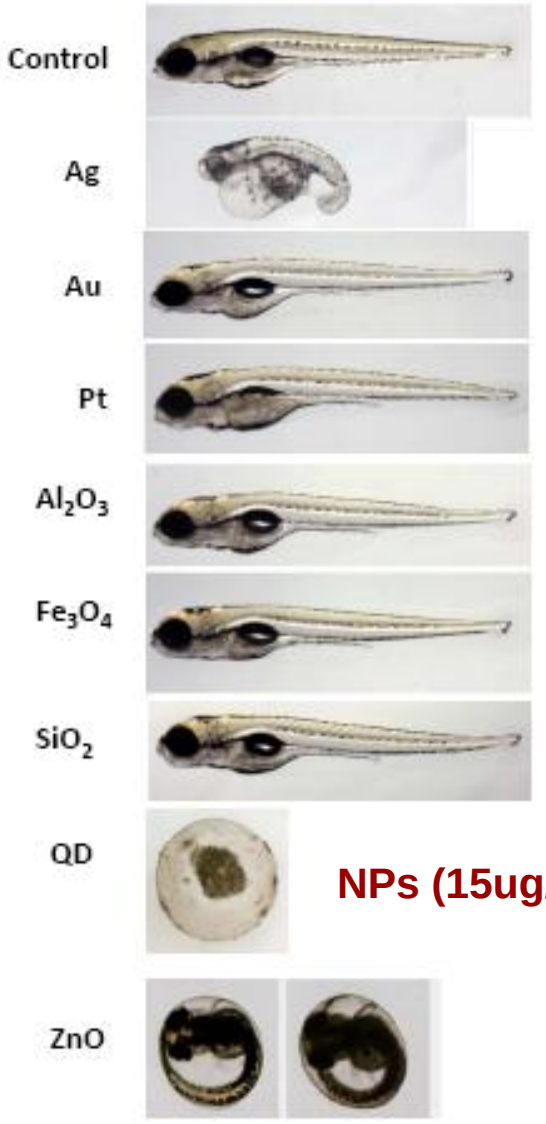


Pearson correlation for each cytotoxicity parameter was calculated from the robust z-score value.

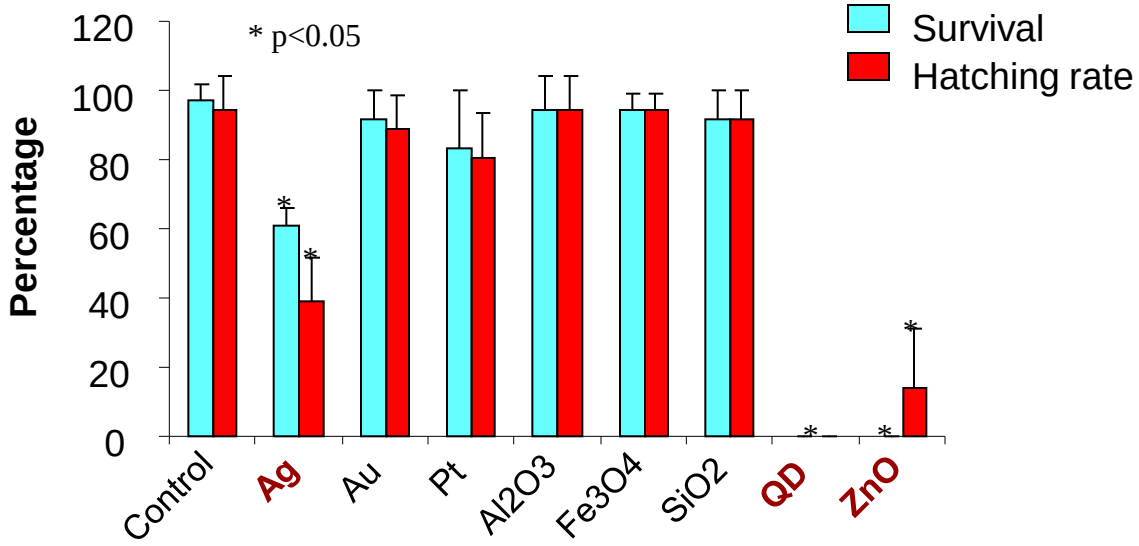
High Throughput DLS of the Kinetics of NP agglomeration in Holtfreter's medium

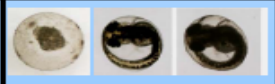


Correlation of HTS results to toxicity screening in in zebra fish



NPs (15ug/mL)

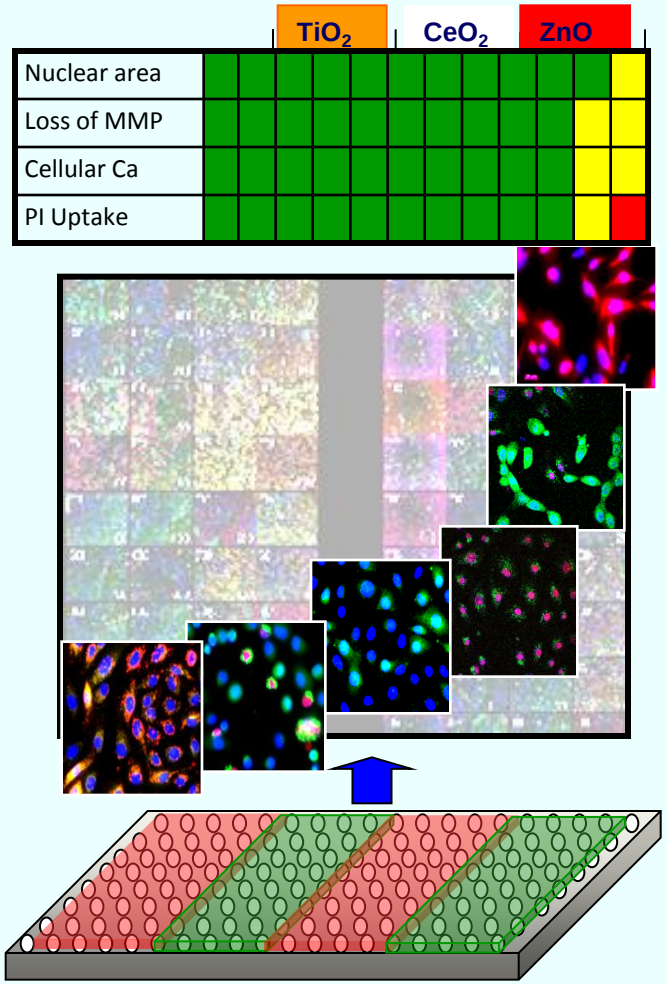


Ranking	NPs	Morphological defects	Physiological defects
0. No morphological or physiological defects	Au, Al_2O_3, Fe_3O_4, SiO_2		
1. Single morphological or physiological defect	-	-	-
2. Multiple morphological and physiological defects	Pt		Low heart beat
3. Severe multiple morphological and physiological defects	Ag		High mortality and reduced hatching rate and low heart beat
4. Embryo do not survive	ZnO, QD		Embryos do not survive or fail to hatch

David Schoenfeld, Tian Xia, Saji George, Shou Lin, Andre Nel

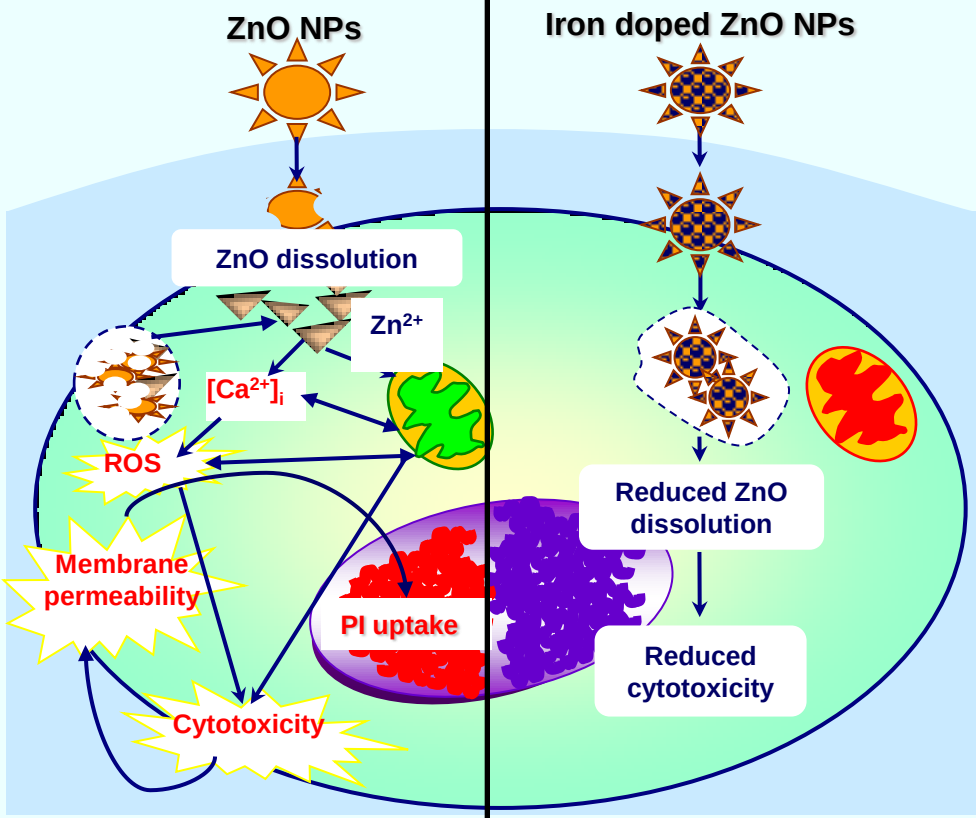
Use of a Predictive Scientific Approach towards Safe design of ZnO

High throughput toxicity screening



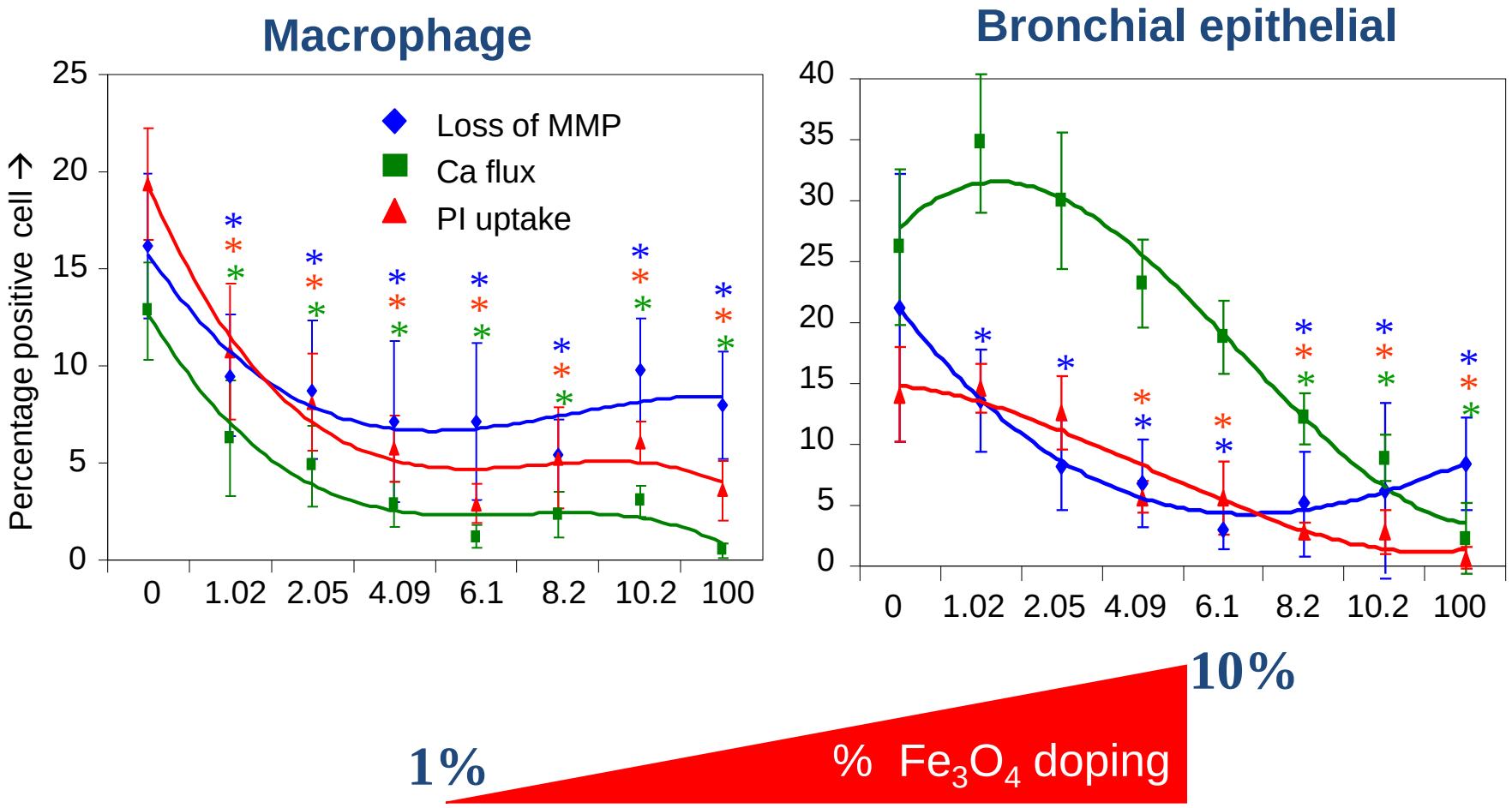
Mechanism of ZnO nanoparticle toxicity

George, S, et al ACS Nano, 2010, 4 (1), pp 15-29.



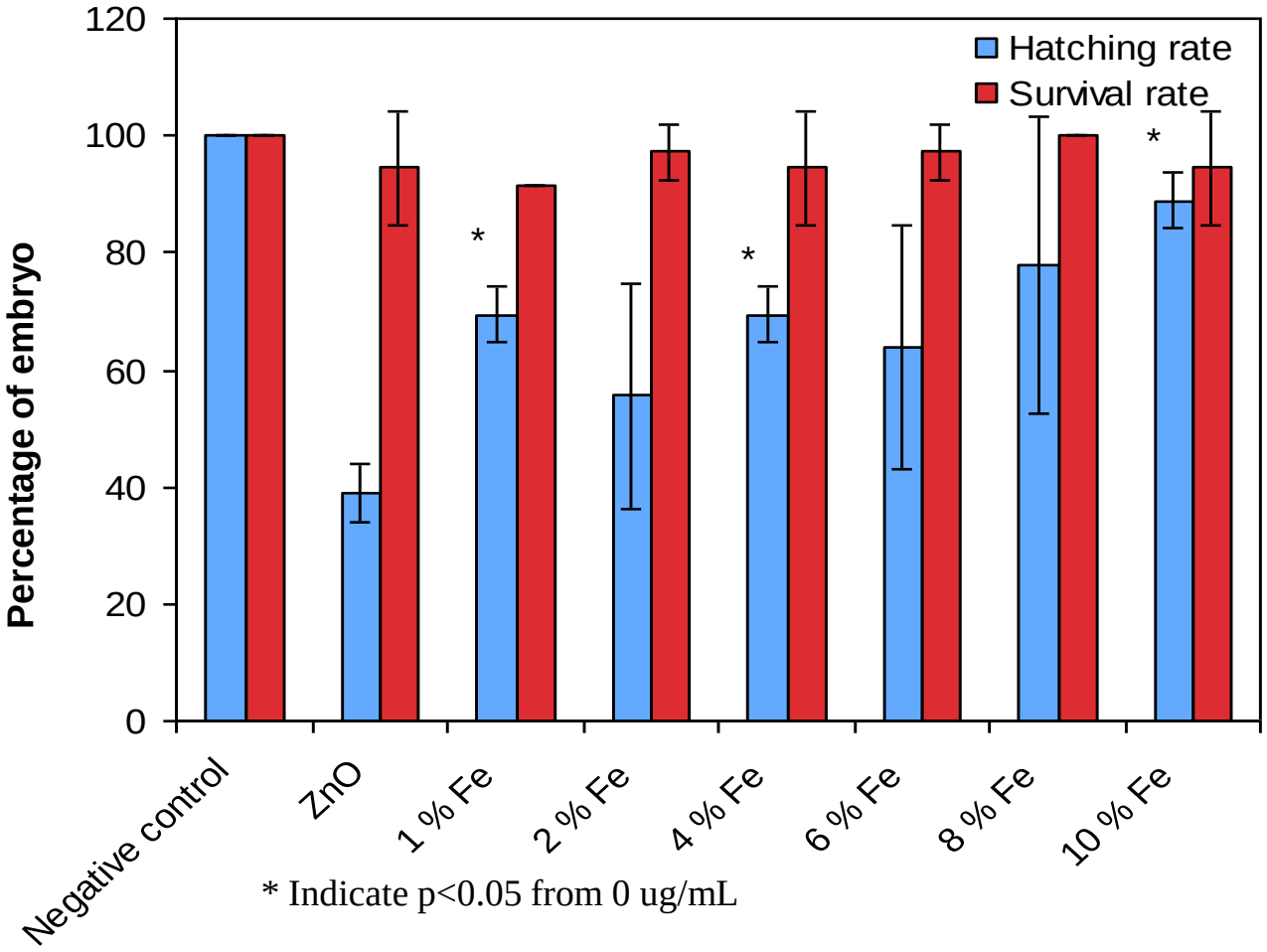
Proposed safe material design

Doped ZnO Nanoparticles are less toxic in HTS analysis

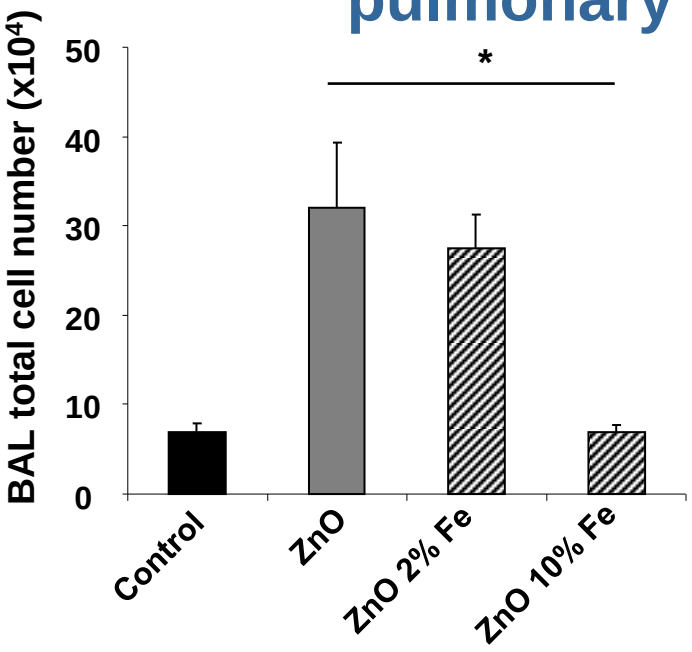


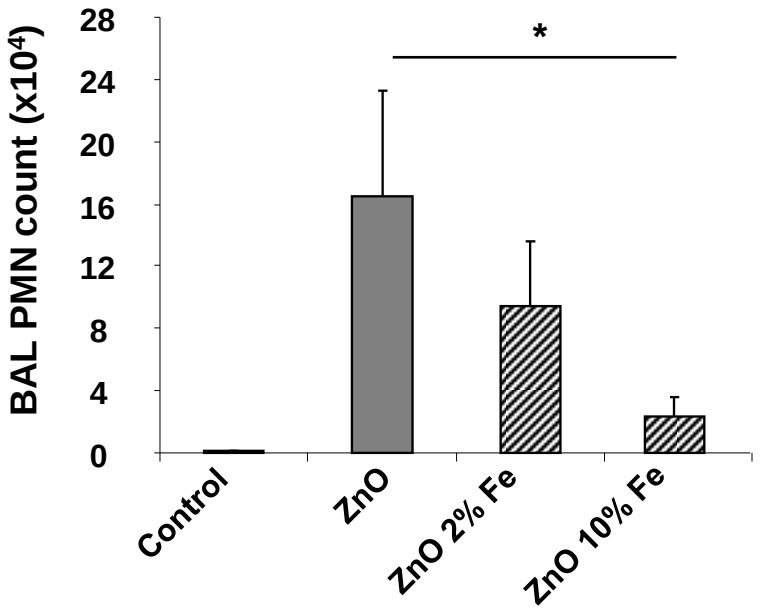
George S, Pokhrel S, Xia T, Ji X, Bradley K, Damoiseaux R, Mädler L, Nel, A E. ACS Nano, 2010, 4 (1), pp 15-29.

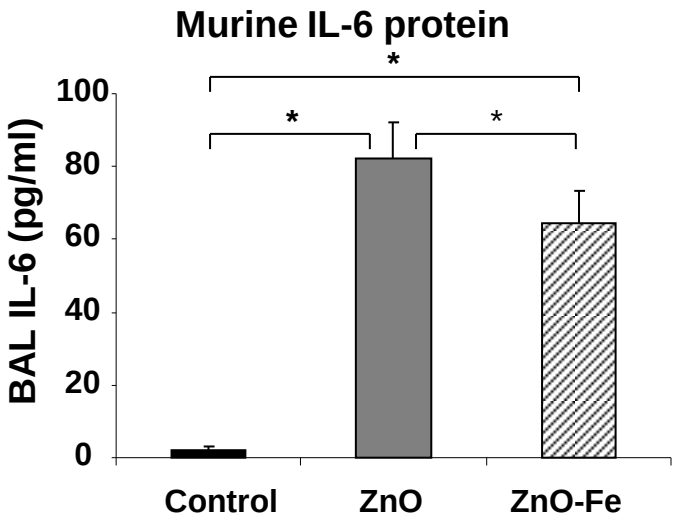
Doped ZnO Nanoparticles are less toxic in Zebrafish Embryo Hatching Experiments

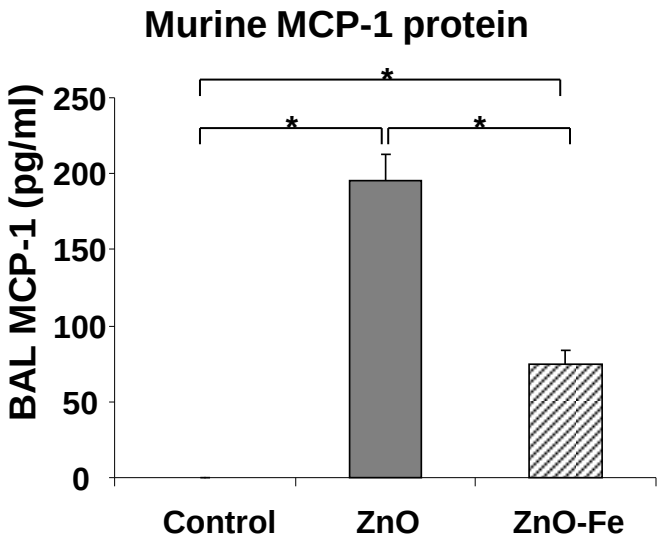


Doped ZnO Nanoparticles are less toxic in pulmonary toxicity in mice



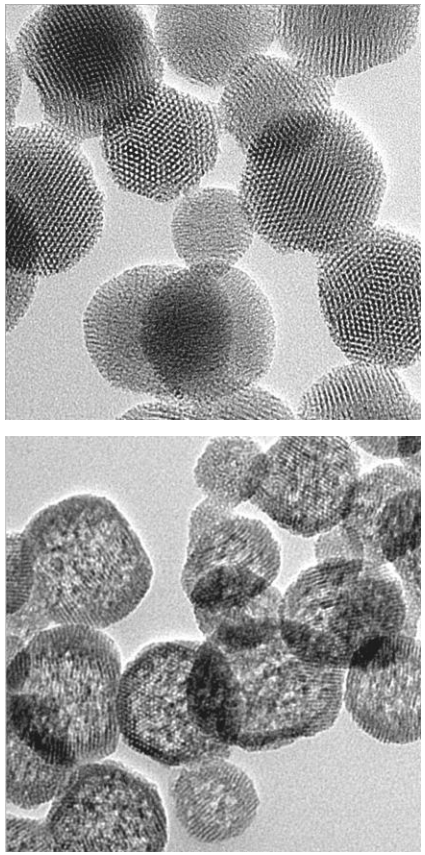






UCLA NanoMachine Center

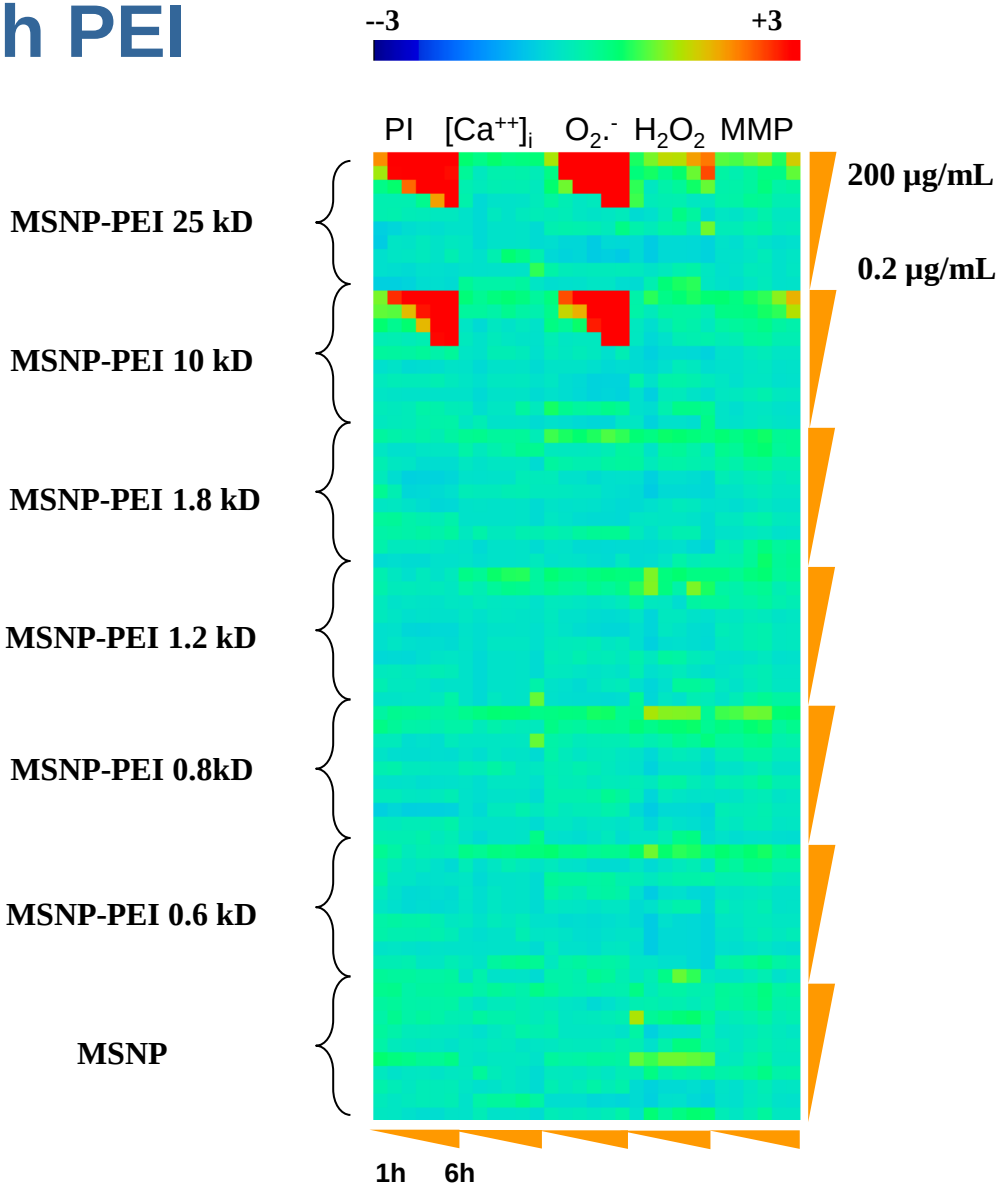
Construction of a cationic MSNP library by coating with PEI



(PEI)

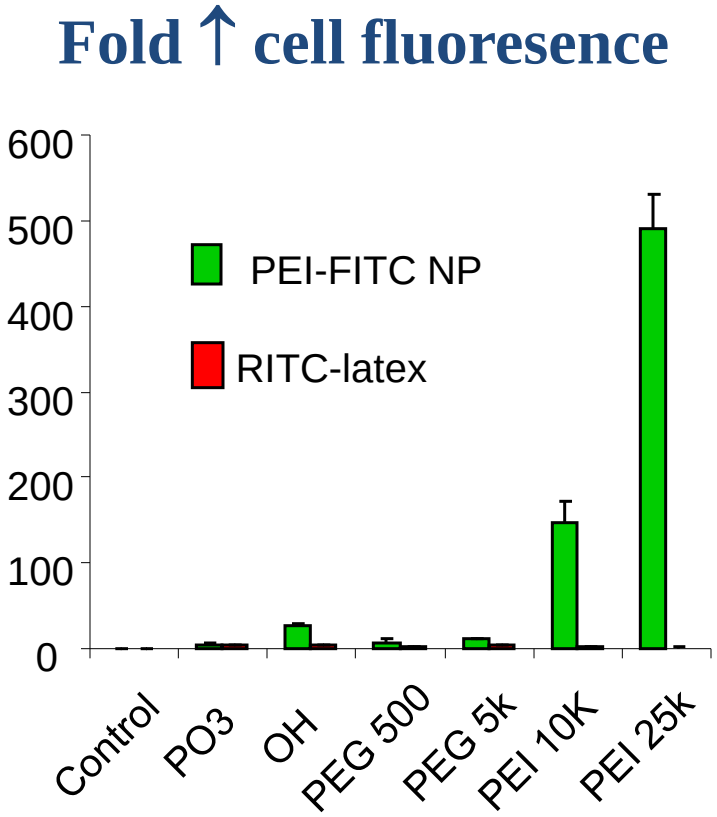
0.6 kD
0.8 kD
1.2 kD
1.8 kD
10 kD
25 kD

Cancer cell lines
NHBE

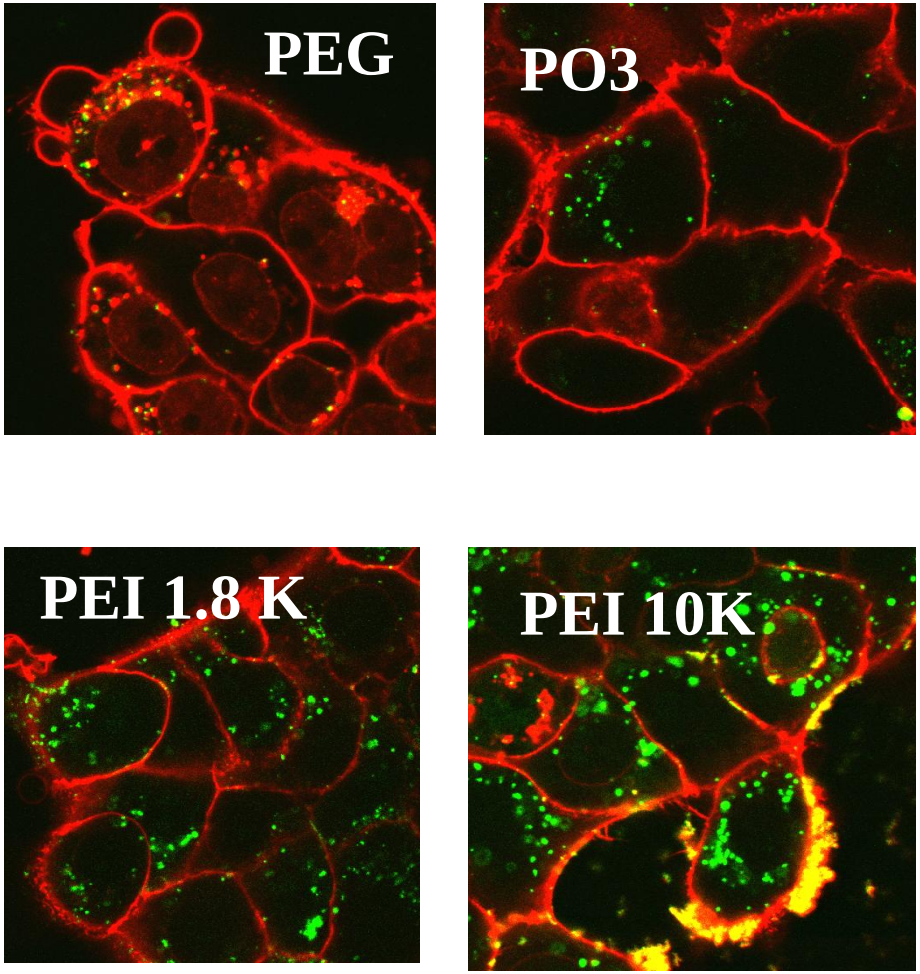


Xia et al. ACS Nano. 2009

Reduced polymer length, low toxicity MSNPs have high uptake in cancer cells

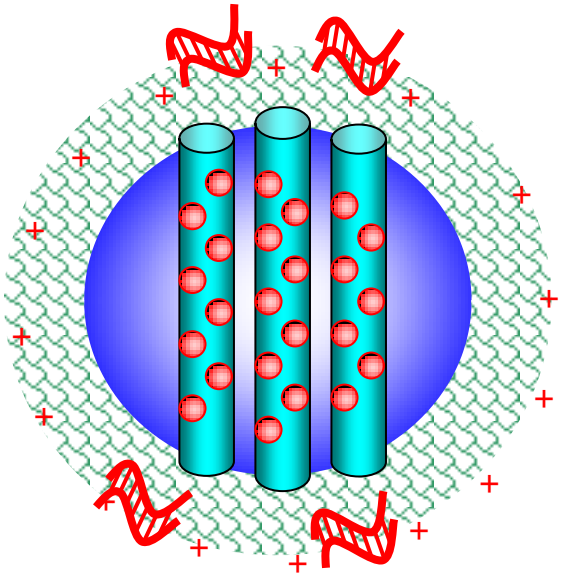


PANC-1 cells



Xia et al. ACS Nano. 2009

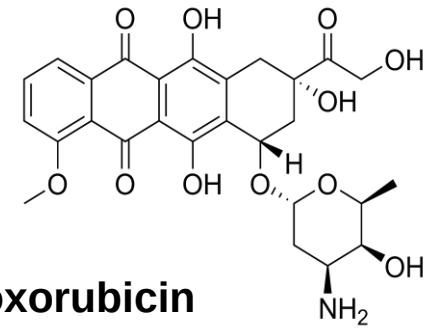
Reduced polymer length MSNPs allow siRNA Attachment but keep the pores available for doxirubicin loading



Doxirubicin

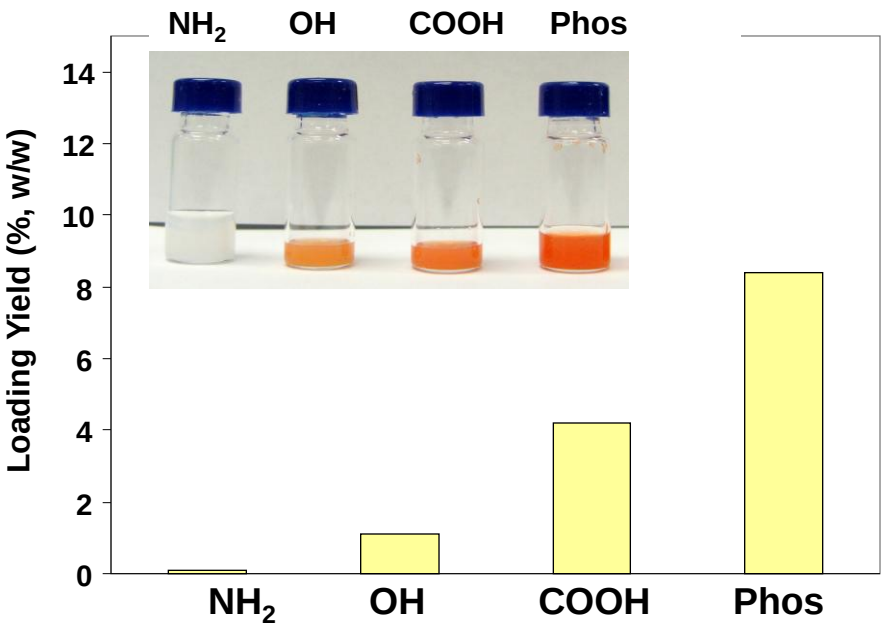
PEI

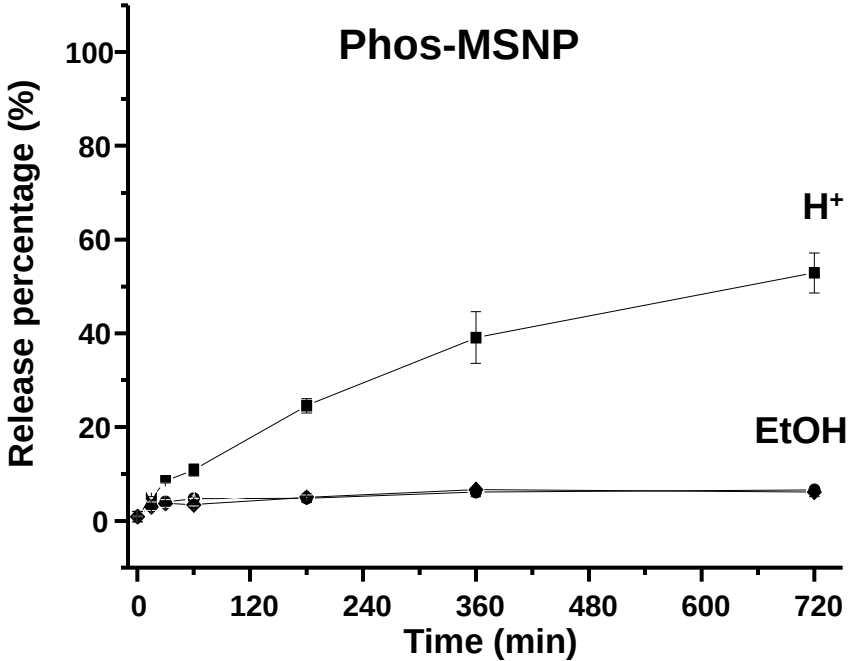
siRNA



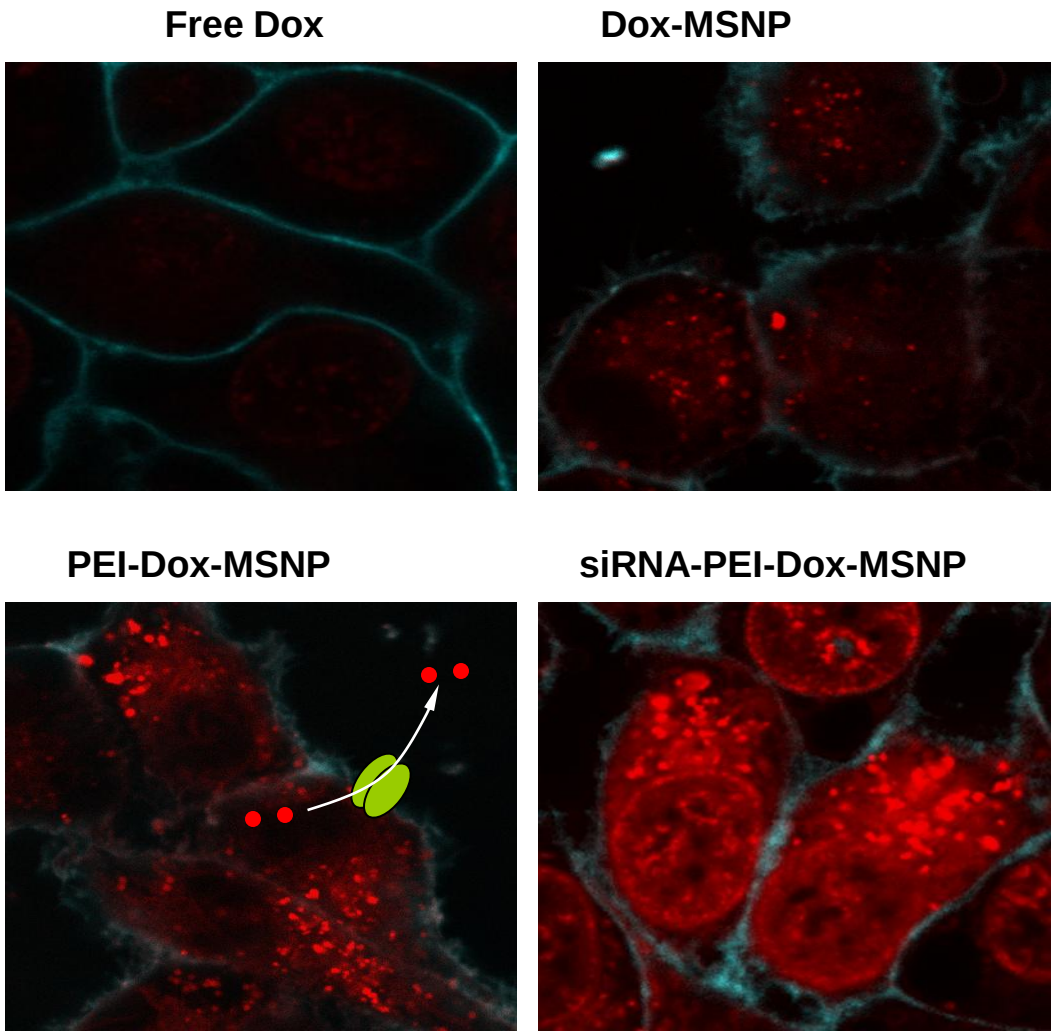
Doxorubicin

Huan Meng et al. ACS Nano. 2010



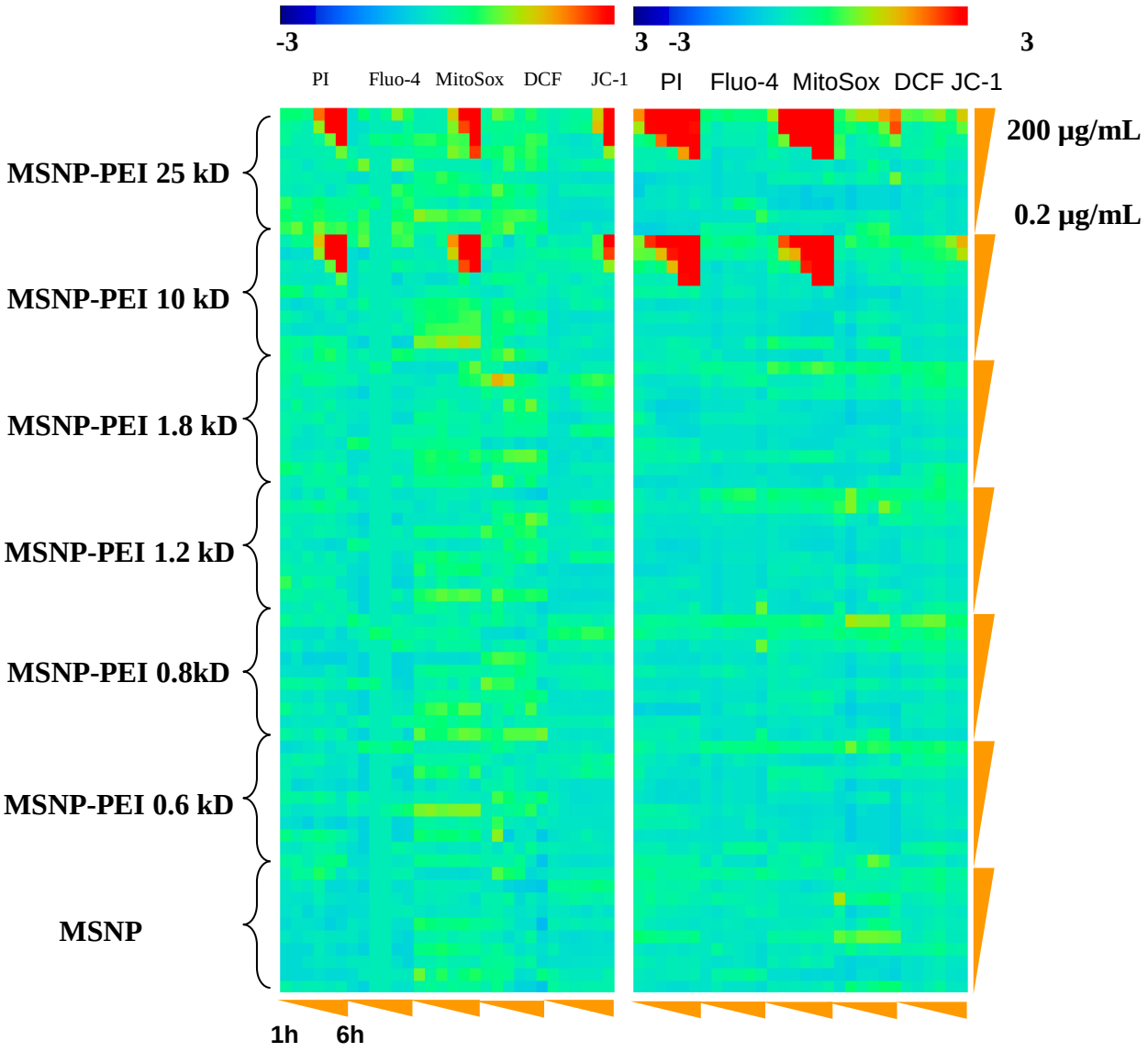


Co-delivery of Pgp siRNA with Dox can Overcome Dox resistance in a Sqaumous carcinoma cell line

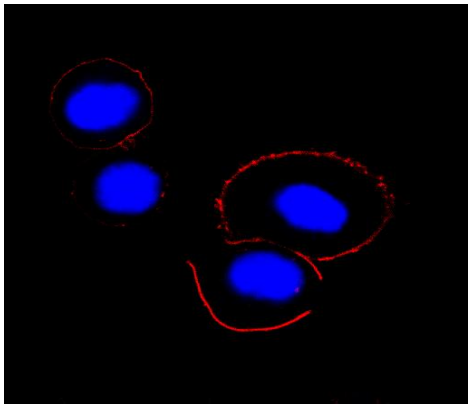


Huan Meng et al. ACS Nano. 2010

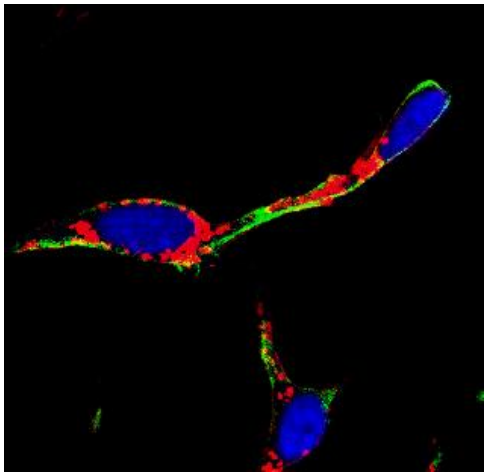
Differential toxicity of cationic NP depending on cellular differentiation



Undifferentiated



Differentiated



Acknowledgements

Nel Laboratory:
Andre Nel
Tian Xia
Saji George
Huan Meng
Xiang Wang
Ning Li
Meiying Wang
Ning Li

Collaborators:
Lutz Maedler
Suman Pohkrel
Jeff Zink
Ivy Ji
Ken Bradley
Robert Damoiseaux
Yoram Cohen
Robert Rallo

Grant support: NSF- and EPA-funded CEIN
NIEHS-funded U19 and RC2